Afferent Synaptic Transmission in a Hair Cell Organ: Pharmacological and Physiological Analysis of the Role of the Extended Refractory Period

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INTRODUCTION

Afferent neuronal discharge in the auditory and lateral line organs contains temporal and intensity information about the stimulus, both represented in modulation of a spontaneous level of discharge (Johnson 1980; Kiang et al. 1965; Rose et al. 1967). Afferent discharge in both organs is stochastic and can generally be described as a modified Poisson point process, with the modification dominated by refractory properties of the fibers (Gaumond et al. 1982, 1983; Gray 1967; Harris and Milne 1966). In this organ, the time course of the extended refractory period is similar regardless of whether the action potential is produced by transmitter release from the hair cell or by antidromic electrical stimulation of the fiber (Harris and Flock 1967). Harris and Milne (1966) suggested that the extended refractory period arises when the firing from one neuromast (the basic organizational unit of the lateral line organ containing a cluster of hair cells) antidromically invades the other neuromasts to reset the firing process. This has been confirmed through subsequent temporal analysis of spike discharge in this organ (Murray and Capranica 1973; Pabst 1976). The presence of a prominent extended refractory period, the ability to characterize the refractory period with antidromic electrical stimulation, and the ability to monitor single unit activity for extended time periods make the Xenopus lateral line organ an ideal subject for pharmacological analysis of the underlying physiological basis of the extended refractory period.

Here we show that drugs that block or enhance afferent neurotransmission modulate the extended refractory period. We have not determined the mechanism by which changes in afferent transmission alter the magnitude of suppression in the refractory period, although one may speculate that voltage-dependent inactivation (with synaptic depolarization) of ion channels active during the refractory period is a reasonable explanation. Because the suppression of discharge during the extended refractory period is attenuated by iberiotoxin and by tetraethylammonium (TEA), two agents known to block calcium-activated potassium channels ($K_{Ca}$), we suggest that these channels may, in part, mediate the extended refractory period.

Our observation that the extended refractory period can be modulated by afferent synaptic input suggests two roles for this process. First, it may extend the response range of the afferent fiber by reducing responses at low stimulus levels. Second, in the lateral line organ, where each afferent fiber branches to innervate different neuromasts, it may serve to self-organize responses by allowing the neuromasts with the strongest signals to dominate the response of the afferent fiber.

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METHODS

The *Xenopus laevis* lateral line organ comprises a series of “stitches” arranged in rows along the body wall. Each stitch contains 3–10 neuromasts, each of which contains 10–30 hair cells, as well as supporting cells. Each neuromast has a cupula that projects outward into the aquatic environment. A single stitch is innervated by two large myelinated afferent fibers. It is possible to place the whole nerve trunk on a wire electrode, destroy all branches of the nerve trunk except to one stitch, and record activity from two single fibers. It is often possible to separate the activity and monitor only one of those fibers if spike amplitudes are different. The whole organ can be easily placed in vitro after removing the piece of skin that contains the neuromasts and its afferent nerve. While it was always possible to isolate activity in the two fibers innervating a single stitch, and often possible to separate the two when spike amplitudes were significantly different, we usually monitored activity in two to four nerve fibers simultaneously to reduce the variability in discharge rate normally seen in spontaneous afferent fiber discharge over time. Monitoring activity in a single nerve fiber was only necessary in analysis of conditional probability. Our methods for monitoring afferent discharge in one or more fibers innervating hair cells in the lateral line organ have been previously published (Bailey and Sewell 2000a; Sewell and Mroz 1987). For this study, we modified slightly our previously published techniques to allow antidromic stimulation through the recording electrode. We repeat these descriptions with modifications below.

Postmetamorphic *Xenopus laevis*, ~2–2.5 cm from nose to vent, obtained from Nasco (Fort Atkinson, WI), were housed at room temperature in de-ionized water containing 1 mM added calcium chloride. Each frog was anesthetized by chilling to near 0°C and decapitated. A piece of skin containing the middle-lateral row of stitches was removed and placed inner surface up on a piece of moistened filter paper. The skin was rinsed with an artificial perilymph solution containing sodium chloride (120 mM), potassium chloride (3.5 mM), calcium chloride (1.5 mM), and glucose (5.5 mM), buffered with HEPES (20 mM), and adjusted to pH 7.5 with sodium hydroxide (total Na+ ~130 mM). Perfusion of the inner surface of the skin allowed relatively rapid diffusion (within 20–40 s) to the basolateral surface of the sensory epithelium. In most experiments, atropine (30 μM) was added to the perfusion medium to eliminate the possible orthodromic activation of cholinergic efferent synapses on the hair cells. In a few experiments, atropine (30 μM) was instead added transiently to assess the presence of efferent activation with antidromic stimulation. All results reported herein represent cases where no efferent responses to antidromic stimulation were evident.

Nerve activity was monitored by dissecting from the inner surface of the skin about 1 cm of the nerve trunk innervating the middle lateral nerve trunk. The freed nerve trunk was sucked into a plastic cone of the skin about 1 cm of the nerve trunk innervating the middle lateral nerve trunk. The observed monophasic action potentials (see Fig. 1, inset) were amplified ~1,000 fold and monitored on an oscilloscope. The signal-to-noise ratio was optimized by analog filtering (high pass, corner frequency of 300 Hz and low-pass, corner frequency of 1,000 Hz). A Schmitt trigger device was used to determine the occurrence of action potentials, which were counted with a microprocessor.

Stimulation and recording of afferent fibers innervating hair cells were carried out alternately through the same chlorided silver wire electrode using a computer-gated switching device. The timing of individual action potentials was determined to microsecond resolution relative to the stimulus pulse with a Tucker-Davis Technologies event timer. Timing of stimulus and gating pulses were controlled by computer with LabView (National Instruments) software. Stimulus pulses were of 140-μs duration at 0.1–10 V in trains of 10 pulses, 3 ms apart. *Time 0* for poststimulus time (PST) histograms was placed at the time of the last stimulus pulse.

Conditional probability was extracted from the interval histogram by dividing the number of events in any time interval by the total number of events in all longer time intervals (Harris and Flock 1967).

Measurement of drug effects required an automated determination of antidromic suppression as a function of time before, during, and after injection of pharmacological agents. Following each antidromic stimulus period, a PST histogram was generated and averaged with the previous 50 antidromic stimulus presentations. From these PST histograms, baseline discharge rate was calculated for the time period 400–500 ms after the start (i.e., 370–470 ms after termination) of the antidromic pulse (long after the refractory period was over). To measure suppression following the antidromic stimulus, a “suppression index” was calculated by averaging the ratio of the discharge rate in each 10-ms bin of the histogram (at poststimulus times from 20 to 110 ms after the termination of the stimulus) to the baseline discharge rate and subtracting the product from 1.

A sigmoidal curve was fit to the data with a least-squares fit of the following equation

\[ y = y_{\text{max}}/\left(1 + \left(\frac{C_{50}}{D}\right)^n\right) \]

where \( D \) is concentration of drug, and \( y \) is the percentage block of suppression. The EC_{50} is the concentration at which the response is blocked by 50%. The factor \( n \) defines the steepness of the curve and is equivalent to the Hill coefficient.

RESULTS

Probability of afferent discharge is reduced following each action potential, whether the action potential is generated synaptically or via antidromic electrical stimulation

Afferent fibers innervating hair cells in the lateral line organ discharge in the absence of mechanical stimulation due to spontaneous, voltage-dependent neurotransmitter release (Sewell 1996). When an electrical pulse or pulse train is applied antidromically through the recording electrode, discharge is suppressed for tens of msec following the antidromic stimulus. The probabilistic nature of the reduction in afferent discharge is shown in Fig. 1, where several traces of afferent discharge are displayed during the time period immediately following an antidromic pulse train. From data such as that shown in Fig. 1, we computed the reduction in probability of discharge following such a shock and presented the data as a PST histogram (Fig. 2).

The suppression following antidromic electrical stimulation can be described as an extended refractory period associated with antidromic conductance of a spike in the afferent fiber. The presence of an extended refractory period in afferent fibers innervating hair cells was first observed nearly 40 yr ago in plots of conditional probability of afferent discharge (Goldberg et al. 1964; Gray 1967). The conditional probability plot can be generated from the interval histogram and indicates the probability of discharge in any given time interval after a previous discharge, provided the nerve has not discharged in earlier time intervals. For a Poisson process, the conditional probability is constant as a function of time. In the lateral line organ, the conditional probability plot deviates from a Poisson process in that following each action potential, there is a reduction in the probability of discharge that lasts >100 ms (Fig. 2; see also Harris and Flock 1967; Harris and Milne 1966). This extended
refractory period is also present in auditory nerve fibers, but is not as pronounced as in the lateral line organ, generally lasting for around 30 ms (Gaumond et al. 1982, 1983; Gray 1967).

In Fig. 2, we compared the time course of the reduction in discharge rate following a single antidromic electrical shock to that of the reduction in probability of discharge following a spike generated via afferent synaptic activation in the same fiber. The time course and magnitude of the extended refractory period is similar regardless of whether the action potential is generated by neurotransmitter released by the hair cell or by antidromic electrical stimulation. The slightly longer period of suppression following an antidromic discharge has been attributed in part to the time required for the action potential generated electrically at the proximal nerve stump to reach the peripheral dendrite (Harris and Flock 1967; Harris and Milne 1966). Because assessment of conditional probability following synaptic activation of discharge requires very long (tens of minutes) data acquisition times in cases where spikes from a
single afferent fiber can be isolated and monitored, most data in this study presented were gathered using antidromic electrical stimulation to generate the extended refractory period.

**Suppression mechanism is probably in the afferent nerve fiber**

The similarity in time course of the extended refractory period seen in conditional probability plots to the period of suppression following antidromic electrical stimulation suggests a common origin for the two phenomena. There are two logical possibilities based on the well-founded assumption that spontaneous discharge in an afferent fiber innervating the lateral line organ is produced by release of neurotransmitter from the hair cell (Sewell 1996). The simplest is that the phenomenon is present in the afferent fiber and represents a reduction in the probability of initiating an action potential following each previous action potential. The second possibility is that the release of a quantity of neurotransmitter sufficient to generate an action potential postsynaptically is followed by a transient reduction in the probability of neurotransmitter release. This requires that antidromic electrical stimulation of the afferent fiber produce a signal that somehow reaches the hair cell. We have considered, and ruled out, several possible mechanisms for this second hypothesis.

The nerve innervating each set of neuromasts in the lateral line organ contains two myelinated afferent fibers and one myelinated efferent nerve fiber. We considered the possibility that the suppression of discharge was due to activation of efferent nerve fibers. Efferent fibers release acetylcholine that activates α-9 nicotinic receptors on the hair cell. Indeed the effect of efferent stimulation on spontaneous discharge bears some similarity to the effects of antidromic stimulation in that both produce a suppression of spontaneous discharge following stimulation (although with efferent activation, the suppression is usually followed by an afterexcitation) (Russell 1971a,b; Sewell and Starr 1991). However, efferent effects can be blocked by perfusion with several cholinergic antagonists known to block the α-9 cholinergic receptor, while antidromic suppression was not blocked by any anticholinergic agent. We did note that, with relatively high stimulus voltage, we occasionally observed an efferent-like response. When efferent-like suppression/excitation was present, perfusion with atropine blocked the efferent-like response, leaving the residual suppression we have characterized as the extended refractory period (Fig. 3). This residual suppression could not be blocked with even very high (1 mM) concentrations of atropine. Thus it appears to be possible, although not common, to activate efferent fibers with a voltage applied through the recording electrode. For all data presented in this paper, we have either perfused the preparation continually with 30 μM atropine (most common) or have injected 30 μM atropine to ascertain the absence of an efferent response to antidromic stimulation.

We also considered the possibility that the extended refractory period may have been mediated through synaptic activation of the hair cell via a mechanism such as release of neurotransmitter from yet-to-be identified efferent nerves or through reciprocal transmission from the afferent fiber to the hair cell, but found no evidence for this. We perfused the lateral line organ with a number of drugs known to block various neurotransmitter receptors. We saw no change in the extended refractory period associated with application of drugs affecting many neurotransmitter receptors, including those for GABA (bicuculline, n = 3), glycine (strychnine, n = 18),...
acetylcholine (atropine, \( n = 27 \) and curare, \( n = 6 \)), cat-
echolamines (phenolamine/alprenalol, \( n = 1 \) each), serotonin
(dihydroergotamine, \( n = 2 \)), CGRP (CGRP, \( n = 2 \)), or
cannabinoid-mediated retrograde transmission (WIN-55
212–2, \( n = 3 \); mesylate, \( n = 5 \); AM-251, \( n = 1 \); AM-630, \( n = 1 \); or AM-281, \( n = 3 \)) (Kreitzer and Regehr 2001; Ohno-
Shosaku et al. 2001; Wilson and Nicoll 2001). The phenomenon
was also unaffected by carbeneoxaline (\( n = 5 \)), an agent
capable of blocking gap junctions (Murray 1955). Thus a
parsimonious hypothesis is that the mechanism to generate the
extended refractory period is located in the afferent fiber and
does not result from transmission of information from the
afferent or an efferent nerve fiber to the hair cell.

Drugs that block calcium activated potassium channels can
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larize afferent fibers, producing “spontaneous” discharge in the
afferent fiber (Sewell 1996). Afferent transmission between the
hair cell and its afferent fiber is mediated by glutamate
(AMPA) receptors (Bailey and Sewell 2000b; Glowatzki and
Fuchs 2002; Niedzielski et al. 1997; Parks 2000; Ruel et al.
1999). We were able to modulate the refractory period with
antagonists and agonists for the glutamate receptor. To block
afferent transmission, we used the glutamate receptor antago-
nist, CNQX. Perfusion of the synapse with CNQX enhanced
the extended refractory period (Fig. 6A) at concentrations that
reduced afferent discharge rate. The enhancement of the ex-
tended refractory period by CNQX was reversible and concen-
tration dependent (Fig. 6B). Similar results were also observed
with another glutamate receptor antagonist, kynurenic acid
(data not shown).

In contrast, depolarization of the afferent terminals by per-
fusion with glutamate receptor agonists reduced the extended
refractory period. Figure 6A shows the ability of AMPA, an
agonist for the AMPA type of glutamate receptor, to reduce the
magnitude of the extended refractory period. A similar action
was seen with low doses of kainate (3–10 \( \mu M \), data not
shown). The attenuation of the extended refractory period is
not likely due to activation of the NMDA subtype of glutamate
receptors because NMDA did not act until concentrations high
equal to stimulate AMPA receptors (0.3 mM) were applied.

Another means of blocking afferent transmission is to block
neurotransmitter release from the hair cell. We were able to
enhance the extended refractory period with agents known to
reduce afferent transmitter release from the hair cell. Cobalt,
which blocks the voltage-dependent calcium channels needed
for transmitter release (Weakly 1973), reduced afferent dis-
charge and enhanced the extended refractory period. Cobalt,
applied at concentrations of 1–1.5 mM (\( n = 7 \)), reduced
afferent discharge rate by 41.3 ± 10.7% (SE) and increased the
suppressing effects following antidromic stimulation (by
37.9 ± 8.5%).

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ated by \( K_{Ca} \) channels is complicated by the presence of
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Extended refractory period can be modulated with drugs
that block or enhance afferent neurotransmission

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vated at resting potentials, and which, if inhibited, would depolarize the hair cell to increase transmitter release and thus discharge rate in the afferent nerve fiber. Indeed, we observed that concentrations of TEA and iberiotoxin that reduced the suppression index of the extended refractory period also increased the spontaneous discharge rate (see Figs. 4D and 7A). This raises the possibility that the reduction in suppression index, produced by these KCa channel blockers, may have been consequent to an increase in afferent synaptic activity rather than a direct action on the afferent fiber. The primary argument against this possibility is that, with perfusion of TEA, it was possible to observe substantial reductions in the suppression index with little or no change in spontaneous activity. An example is shown in Fig. 7B, where relatively large, dose-dependent reductions in suppression index are seen in the absence of any change in spontaneous discharge rate. Indeed, such observations were not uncommon, as can be observed in Fig. 7C, where we have plotted the relation between the change in spontaneous discharge to the drop in suppression index for each TEA perfusion. Here are numerous cases where large changes in suppression index are accompanied by relatively small increases or small decreases in spontaneous discharge.
rate. In addition, there was no strong correlation ($r^2 = 0.058$) between the increase in spontaneous discharge rate and the reduction in the extended refractory period following TEA administration (Fig. 7C), indicating that only a small fraction of the TEA-mediated change in suppression index can be attributed to the TEA-mediated change in spontaneous rate.

**DISCUSSION**

The extended refractory period observed in spontaneous discharge of afferent fibers is remarkably similar in time course to that observed following antidromic electrical stimulation of the nerve trunk (Harris and Flock 1967), suggesting that the extended refractory period may be generated by antidromic invasion of spontaneously generated action potential from one afferent nerve branch to others. The preponderance of evidence suggests the refractory period is generated through activation of potassium channels. Much of the refractory period is susceptible to agents capable of blocking calcium-activated potassium channels (TEA and iberiotoxin), although there is a smaller component susceptible to 4-aminopyridine, which may be blocking A-type potassium currents prevalent in auditory neurons in vitro (Jagger and Housley 2002).

We have shown that drugs that alter glutamatergic transmission between the hair cell and its afferent fiber modulate the extended refractory period in afferent fibers of the lateral line organ. Drugs that reduce transmission, such as glutamate receptor antagonists and agents that block voltage-dependent transmitter release, enhance the extended refractory period. Glutamate receptor agonists, which effectively enhance synaptic strength by depolarizing the afferent fiber, reduce the extended refractory period. These observations led us to suggest a functional role of the extended refractory period in regulating synaptic strength whereby weak synaptic signals are self-suppressed.

An ability to modulate the extended refractory period with afferent synaptic input could accomplish an expansion in the response range even when there is only a single synaptic input (as is the case in the mammalian auditory system) (Liberman 1982). The presence of an extended refractory period following any spike generated in the fiber will self-suppress the proba-
bility of a subsequent discharge. When synaptic input is weak, such as with small displacement of the stereocilia, this self-suppression of subsequent discharge will be greater than when synaptic input is strong. An obvious implication is that this phenomenon should amplify the response range for synaptic transfer of signal at the afferent synapse by reducing the probability of discharge when transmitter release from the hair cell is low (Fig. 8A). This is advantageous in a synapse that must carry intensity information about the sensory stimulus while preserving temporal information. This phenomenon could be dynamic within a single synapse in that if synaptic input changes, the magnitude of the extended refractory period will change.

A second useful feature is that modulation of the extended refractory period should also enhance temporal precision at low stimulus levels, at least for signals where significant information is contained within the extended refractory period. Because the probability of discharge is reduced in the extended refractory period, discharge may be biased to occur only during the temporal peak of the stimulus.

The prominence of the extended refractory period in the discharge pattern in afferent fibers of the lateral line organ suggests an additional role for the phenomenon. The modulatable extended refractory period may act as a self-organizing mechanism for processing converging afferent input. In the lateral line organ, afferent fibers branch extensively to innervate many hair cells. In this nerve, action potentials generated at each branch point back-propagate down the other branches (Murray and Capranica 1973; Pabst 1976). Each back-propagated action potential produces an extended refractory period like that produced via a synaptically generated action potential (Fig. 8B). A synapse with strong synaptic input will be less susceptible to suppression by spikes generated at other synapses innervated by the same afferent fiber. In other words, the strong synapses become stronger and the weak become weaker. This mechanism will allow one synapse with a particularly strong input to dominate the integrated response of the fiber, but allows for integration of all responses when there is no particularly strong input. The system should be self-organizing in that synapses with stronger synaptic inputs will actively suppress those with weaker synaptic inputs. However with any dynamic change in input strength, the system could self-adjust to expand to the greatest possible response range.

A similar phenomenon has been observed elsewhere in the nervous system. One of the first descriptions of this extended refractory period comes from analysis of discharge patterns in neurons in the superior olivary complex (SOC) (Goldberg et al. 1964), in which conditional probability plots were obtained at

**FIG. 7.** Although TEA increased spontaneous discharge rate and decreased extended refractory period (as indicated by a reduction in the suppression index) over similar dosage ranges (A), change in discharge rate did not correlate well with drop in suppression index (B and C). A: decrease in suppression index (○) occurred over the same dosage range as the increase in spontaneous discharge rate (□), although at concentrations over 1 mM, the effects of TEA on spontaneous discharge were highly variable (mean ± SE are plotted). Data and curve fit for the effects of TEA on the suppression index are shown as those plotted in Fig. 4C. B: ability of TEA to reduce the suppression index was not well correlated to change in spontaneous rate. An example of the ability of TEA to block the suppression index (bottom) without significantly changing spontaneous discharge rate (top) is shown. Time scale is indicated with the heavy bar (500 s) in the bottom panel. Discharge rate is taken at times 370–470 ms after stimulus as described in Fig. 4. C: change in spontaneous rate and the suppression index during TEA perfusion is plotted for each individual perfusion. Note the preponderance of points for which there is a substantial decrease in the suppression index, while spontaneous discharge was either suppressed or relatively unaltered. Linear regression analysis shows little correlation ($r^2 = 0.058$).
different stimulus intensities. Interestingly, the extended refractory period in these SOC neurons decreased with increased stimulus intensity, as we predict would be the case if our results were applicable to those neurons.

While it is appealing to ascribe a functional role for the extended refractory period in stimulus localization in the lateral line organ, we do not think this is the case. Interactions that modulate the extended refractory period take place within a single stitch. Each stitch is around 2–3 mm in length. For the phenomenon to play a functional role in lateral directional orientation, the stimulus signal in the water would need to attenuate over that 2–3 mm length of the stitch so that the neuromasts nearer the signal could dominate the others. However, the attenuation of a sound wave in water over that distance is negligible. Nor would temporal considerations appear to be important since the travel time of the wave over that distance is insignificant (at well over 1,000 m/s, the wave has traveled the length of the stitch in about a microsecond) compared with the length of the extended refractory period (tens of milliseconds) and the characteristic frequencies of these fibers (16–32 Hz).

Our demonstration that the extended refractory period is modulated by glutamate receptor ligands suggests the phenomenon can normally be regulated by release of excitatory transmitter from the hair cell. Glutamate receptor ligands could modulate the extended refractory period through a change in voltage in the postsynaptic terminal with changes in synaptic input. Exogenous glutamate receptor agonists and increased transmitter release from the hair cell both depolarize the afferent nerve terminal. Conversely, exogenous antagonists or decreased transmitter release both hyperpolarize the terminal (by reducing the depolarization due to resting transmitter release).

In addition to activating antidromic spikes in the afferent nerve fibers, stimulation of the nerve trunk can also activate efferent nerve fibers, which synapse on the hair cells. However, the modulatable extended refractory period is not likely due to efferent activation. All of the effects observed with efferent stimulation can be blocked by antagonists of the α-9 nicotinic acetylcholine receptor (Russell 1971a; Sewell and Starr 1991), whereas the suppression we observe in this study cannot. In this study, we used atropine, either applied to the perfusate constantly or transiently, to rule out the presence of a cholinergic effect. Furthermore, efferent effects occur at higher stimulus voltages than those used in this study (data not shown; also see Gorner 1967), consistent with the much smaller diameter of efferent fibers compared with afferent fibers.

The extended refractory period was substantially blocked by TEA and iberiotoxin. We suggest it may be mediated by activation of a KCa channel, probably of the BK type at the level of the afferent fiber. However, BK channels are known to be present in hair cells and likely activated at resting potentials (Fettiplace and Fuchs 1999). Blocking those channels could depolarize the cell to increase transmitter release and afferent discharge rate. TEA and iberiotoxin both increase the discharge rate in afferent fibers, an action consistent with such an effect. Thus at least part of the effect of BK channel blockers to reduce the extended refractory period is likely due to an action on the hair cell to increase transmitter release. However, it was often possible to observe changes in suppression index without changes in discharge rate. In addition, the magnitude of the increase in spontaneous discharge rate did not correlate very well with the amount of reduction of the enhanced refractory period. Thus it is plausible that there is an additional site of action KCa blockers in attenuating the extended refractory period. A direct action on the afferent fiber would atten-
uate the extended refractory period if it is mediated in part by BK channels; an action to depolarize the hair cell and increase transmitter release could also attenuate the refractory period. The contribution of the latter action would depend on the state of the hair cell and the level of intrinsic activation of its BK channels.

A role for BK channels in mediating a part of the extended refractory period is consistent with its action in other neuronal systems, where it has been shown to contribute to repolarization of the action potential and to contribute to afterhyperpolarization following the action potential (Cloues and Sather 2003; Edgerton and Reinhart 2003; Pedarzani et al. 2000; Shao et al. 1999; Zhang et al. 2003). Also relevant to our finding is the demonstration that BK channel activation in dorsal root ganglion cells can suppress action potential firing (Zhang et al. 2003). Finally, large conductance K\(_{\text{Ca}}\) channels have been described in a subpopulation of auditory neurons in the mouse (Adamson et al. 2002) and in saccular neurons from the mouse (Adamson et al. 2002) and goldfish (Davis 1996), providing some support for the idea of a role of these channels in the extended refractory period.

We speculate that the ability to modulate the extended refractory period with glutamate receptor ligands and with blockers of voltage-dependent transmitter release is consistent with a mechanism for the extended refractory period involving BK channels on the afferent nerve terminal. Some BK channels can inactivate with voltage (Solaro et al. 1995), if appropriate \(\beta\)-subunits are present in the channel (Armstrong and Roberts 2001). Thus depolarization of the terminal by synaptic activity might inactivate the channels, while blocking ongoing synaptic input with glutamate receptor antagonists could reduce voltage-dependent inactivation.

The question of how the action potential activates the K\(_{\text{Ca}}\) channel is not answered, although activation of a voltage-dependent calcium channel in the afferent terminal to increase intracellular calcium would be a logical choice. A simple mechanism for the extended refractory period thus might be that an action potential in the afferent nerve terminal, whether generated synaptically or by antidromic electrical stimulation, can activate a K\(_{\text{Ca}}\) (BK) channel via voltage-dependent calcium entry to reduce the probability of occurrence of a subsequent action potential for a period of <100 ms.

The mechanism we describe is general and could serve a similar role elsewhere in the nervous system. All that is required is K\(_{\text{Ca}}\) channel and excitatory synaptic input. It provides a simple means to reduce input from synapses where signals are small, but allows for dynamic changes in the integration of input when presynaptic signal-strength changes. Within a single synapse, this strategy amplifies the response range for a given range of stimulus intensities. In neurons with multiple afferent inputs, such as in the lateral line organ, it provides a simple means of self-organizing inputs such that a maximum response range is available across all inputs.

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**References**


Bailey GP and Sewell WF. Contribution of glutamate receptors to spontaneous and stimulus-evoked discharge in afferent fibers innervating hair cells of the Xenopus lateral line organ. Hear Res 144: 8–20, 2000b.


