The Reversal Potential of the Spike Afterhyperpolarization in Bullfrog Sympathetic Neurons

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Summary: Intracellular recordings were made from neurons in bullfrog sympathetic ganglia maintained in vitro. The action potential afterhyperpolarization was comprised of two components, namely an initial, shorter-lasting (up to 200 ms) event and a later, longer-lasting (up to 2500 ms) one, both of which resulted from an opening of potassium channels. Both of these components reversed their polarities at between -80 and -100 mV. The initial part of the afterhyperpolarization, however, always reversed its polarity at a less negative potential than the latter part. Mathematical analysis, by means of a hypothetical membrane equivalent circuit, revealed that this difference in the reversal potentials could be explained by a simple potassium-channel opening hypothesis without considering any additional current other than potassium-current for either component of the spike afterhyperpolarization.

Key words: calcium-dependent potassium conductance—action potential afterhyperpolarization—caffeine-induced hyperpolarization—sympathetic neurons—bullfrog

Introduction

An afterhyperpolarization which follows a single or repeated action potential(s) is calcium-dependent in bullfrog sympathetic neurons, as in many other central and peripheral nerve cells (Meech, 1972, 1978). A widely accepted concept of this calcium-dependent hyperpolarization is that calcium ions enter the neuron as a charge carrier during the action potential and cause a transient increase in intracellular calcium concentrations and, thereby, open potassium-channels (Meech, 1972, 1978). The opening of potassium-channels or the activation of membrane potassium conductance ($g_K$) by intracellular calcium ions brings the membrane potential to the equilibrium potential for potassium ions ($E_K$). The reversal of the response should, therefore, occur at potentials more negative than $E_K$.

Earlier work in bullfrog ganglia has shown that the spike afterhyperpolarization comprises two components, an initial, shorter-lasting and a slower, longer-lasting part. The initial component of the spike afterhyperpolarization reverses its polarity at a less negative potential than the latter component (Kuba et al. 1983; Tokimasa, 1984a). The present study was performed to examine the reasons for these two components reversing at different potentials.

Materials and Methods

Bullfrog (Rana catesbiana) sympathetic ganglion (9th or 10th) with its associated sciatic nerve was continuously perfused
with Ringer solution of the following composition (mM): NaCl 112, KCl 2.0, CaCl₂ 1.8 and NaHCO₃ 2.5. The preparation was pretreated with collagenase (1%) (Sigma type 2) for 60 minutes prior to the experiment in order to loosen the connective tissue. Either fast B (Dodd and Horn, 1983; Tokimasa, 1984a) or slow B (Dodd and Horn, 1983; Tokimasa, 1984a) neurons were impaled with a glass microelectrode containing 3M KCl (DC resistance 15–50 MΩ). Other basic techniques for the stimulation of the sciatic nerve, cell classification and intracellular current-injection are detailed elsewhere (Nishi and Koketsu, 1960; Dodd and Horn, 1983; Tokimasa, 1984a, b). The soma action potential which was observed in response to sciatic nerve stimulation or to intracellular current-injection is, respectively, referred to as an antidromic or a direct spike in this paper. Drug used was caffeine (Wako pure chemical). All the experiments were carried out at room temperature (20–24 °C).

Results

The observed reversal potential of the spike afterhyperpolarization

In general, the resting membrane potential of bullfrog ganglion cells was between -60 and -70 mV (Fig. 1). A single soma action potential (overshoot; +30–+35 mV) was followed by a prolonged afterhyperpolarization, which usually lasted for more than 500 ms up to 2500 ms (Fig. 1). The spike afterhyperpolarization reached an initial peak immediately after the spike repolarization. This was followed by a sec-

![Fig. 1. The initial and the second peaks of the spike afterhyperpolarization. Upper trace; a direct spike observed on a fast time base. Lower trace; a spike afterhyperpolarization of the same cell observed on a slower time base. Full height of the action potential is not shown. The afterhyperpolarization had an initial peak (-80.5 mV) immediately after the spike repolarization and a second peak (-86.3 mV) 140 ms after the initial peak. In many other cells tested, the temporal separation of two peaks was less clear than that shown in this particular cell (see Figs. 2, 5 and 6). The resting potential was -60 mV and the spike amplitude was 90 mV. These traces were displayed on the chart via a digitizing oscilloscope.](image-url)
ond peak, at 50 to 150 ms after the initial peak, and this declined monotonically thereafter (Fig. 1). This after hyperpolarization comprises two components, an initial, shorter-lasting (up to 200 ms) part and a slower, longer-lasting (up to 2500 ms) part (Tokimasa, 1984a). It has been proposed that the initial and the latter part are generated by a delayed rectifier $g_K$ ($g_{KDR}$) and a calcium-dependent $g_K$ ($g_{KCa}$) (Kuba et al. 1983; Tokimasa, 1984a), respectively.

The clear reversal of the afterhyperpolarization occurred at potentials more negative than -100 mV (Kuba et al. 1983; Tokimasa, 1984a); -80.6 mV and -87.5 mV have been reported by Kuba et al. (1983) as the null potentials for the initial and the second peaks, respectively. Quite comparable null potentials (-89 mV and -93 mV) have also been reported (Tokimasa, 1984a). In a given neuron, however, the initial peak always reversed its polarity at a less negative potential than the second peak (Fig. 2 and Table 1). There could be several possibilities which may explain such a difference. For example, this could mean that either or both contains another current component in addition to $g_K$ (Kuba et al. 1983). In other words, the equilibrium potential of either one (or both) is slightly shifted from $E_K$. On the other hand, the reversal potential of two components was shifted by 57 mV for one decade change in extracellular potassium concentration (Fig. 3), indicating that probably neither the initial nor the later component of the afterhyperpolarization (AHP) contains additional currents. For this reason, both of these components are tentatively assumed to be "purely" potassium dependent in the following mathematical analysis.

![Fig. 2. Two different reversal potentials. The soma membrane was hyperpolarized from the resting potential (-70 mV) to -85 mV by constant DC-current. The initial peak of the spike afterhyperpolarization nulled at this potential (indicated by an arrow) but the latter component had not yet been nulled. The null potential of the second peak was -90 mV. Inset shows the action potential which was observed at the resting potential. The action potentials were generated in response to the antidromic stimulation applied to the sciatic nerve.](image-url)
TABLE 1  
Two different reversal potentials

<table>
<thead>
<tr>
<th>Experiments</th>
<th>$E_{\text{AHP(fast)}}$</th>
<th>$E_{\text{AHP(slow)}}$</th>
<th>$E_{\text{AHP(fast)}} - E_{\text{AHP(slow)}}$</th>
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<td>1</td>
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<td>$-101$ mV</td>
<td>8 mV</td>
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The reversal potential of the spike afterhyperpolarization ($E_{\text{AHP}}$) was measured in 21 cells. $E_{\text{AHP(fast)}}$ denotes the reversal potential of the initial peak of the afterpotential. $E_{\text{AHP(slow)}}$ denotes the reversal potential of the second peak which was measured from 50 to 150 ms after the initial peak. The average difference between $E_{\text{AHP(fast)}}$ and $E_{\text{AHP(slow)}}$ in these cells was $5.3 \pm 0.6$ mV (mean ± SE, mean, $n=21$). Paired Student 't'-test revealed that there was a significant difference between these two reversal potentials ($<0.001$).

The predicted reversal potential of the spike afterhyperpolarization

Fig. 4 represents a hypothetical membrane equivalent circuit used in the present study. $g_{\text{Na}}$ represents the resting sodium conductance. This conductance can be activated during the action potential but the activation is transient (less than a few ms) since concomitant inactivation rapidly occurs. $g_{\text{K}}$ represents the resting potassium conductance. It is possible that this resting $g_{\text{K}}$ is identical to the conventional delayed rectifier $g_{\text{K}}$ but there is yet no evidence which has clearly demonstrated that these two conductances are identical. For this reason, a resistor $1/g_{\text{K}}$ is separated from $1/g_{\text{KAHP}}$ in the circuit. Calcium conductance ($g_{\text{Ca}}$) is also activated during the action potential. An residual increase in $g_{\text{Ca}}$ would be a possible current component which contaminates the afterpotential. If this were the case, an inward current through a resistor $1/g_{\text{Ca}}$ would cause an inward (depolarizing) shift in the reversal potential of the spike afterhyperpolarization. The initial peak of the spike afterhyperpolarization, however, was not changed by calcium channel blockers, such as cobalt or manganese (Tokimasa, 1984a), findings which indicate that the residual
**Fig. 3.** Potassium dependence of $E_{\text{AHP}_{\text{slow}}}$.

A: the spike afterhyperpolarization at various membrane potentials (indicated besides each trace) in Ringer solution which contained 2 mM (left), 3 mM (middle) or 5 mM (right) potassium ions. B: the afterhyperpolarization of the same cell as in A at -96 mV in normal Ringer solution (2 mM K). Note the clear decrease in input resistance at the null potential of the slow component (indicated by arrows). C: $E_{\text{AHP}_{\text{slow}}}$ in various potassium concentrations. Each circle and bars denote the mean and standard error of the mean. The number of experiments are indicated in each parenthesis. The regression line had a slope of 57 mV for one decade change in potassium concentration. Full height of the action potentials are not shown in A and B.

**Fig. 4.** The hypothetical membrane equivalent circuit for calculation of the predicted reversal potential of the spike afterhyperpolarization. Variable resistor $1/g_{\text{KAHP}}$ denotes the sum of the delayed rectifier potassium conductance and the calcium-dependent potassium conductance. Switch $S_1$ was assumed to be closed during the action potential. $I$ denotes the constant-current source switched on-off by $S_2$. See also the text for detail.

Increase in $g_{\text{Ca}}$ does not significantly participate in the spike afterhyperpolarization. $g_{\text{Ca}}$ was, therefore, neglected in the present analysis. A variable resistor $1/g_{\text{KAHP}}$ is defined as the sum of $g_{\text{KDR}}$ and $g_{\text{KCa}}$; all potassium-currents which participate in the afterhyperpolarization. If we assume that $g_{\text{Na}}$ and $g_{\text{K}}$ are not voltage-dependent at between -60 and -100 mV, and that $g_{\text{K}}$ can be estimated from small hyperpolarizing electrotonic potentials, then the potential change resulting from closing $S_1$ could be calculated as follows:

$$
g'_{\text{K}} = g_{\text{K}} + g_{\text{KAHP}} = \alpha \cdot g_{\text{K}} (\alpha > 1) \quad \text{Eq (1)}$$

$$
g_{\text{KAHP}} = g_{\text{KDR}} + g_{\text{KCa}}$$

When $S_1$ is closed, current ($i'$) can be calculated by Kirchoff's law in that

$$
i' = \frac{E_{\text{Na}} - E_{\text{K}}}{1/g_{\text{Na}} + 1/(\alpha \cdot g_{\text{K}})} \quad \text{Eq (2)}$$
Membrane potential changes from the resting potential ($E_R$) to $E'_r$ where

$$E'_r = E_K + \frac{i'}{\alpha \cdot g_K} \quad \text{Eq (3)}$$

When $S_2$ is closed and $S_1$ opened, constant-current ($i_m$) crosses the membrane which moves the potential from $E_R$ to $E_m$.

Therefore,

$$i_m = (E_m - E_r) \cdot g_K \quad \text{Eq (4)}$$

If $S_1$ is closed with $S_2$ closed, the total membrane current through $\alpha \cdot g_K$ becomes ($i' + i_m$). Therefore, the potential moves from $E_m$ to $E'_r$ where

$$E'_r = E_K + \frac{i' + i_m}{\alpha \cdot g_K} \quad \text{Eq (5)}$$

That is to say, the amplitude of the spike afterhyperpolarization ($V$) is

$$V = E'_r - E_m = E_K + \frac{i' + i_m}{\alpha \cdot g_K} - E_m \quad \text{Eq (6)}$$

We cannot measure the membrane current but can substitute for $i'$ from Eq (2) and for $i_m$ from Eq (4). Whence,

$$V = E_K + \frac{1}{\alpha \cdot g_K} + \frac{1}{\alpha \cdot g_K} \cdot \frac{E_m - E_K}{E_m - E_r} \cdot g_K$$

$$= E_K - E_m + \frac{g_Na \cdot (E_m - E_K)}{\alpha \cdot g_K + g_Na} \cdot \frac{E_m - E_r}{\alpha} \quad \text{Eq (7)}$$

Eq (7) can be used to predict the amplitude of the spike afterhyperpolarization from conductance change $\alpha$ for any given potential levels.

The results are illustrated in Fig. 5. The predicted reversal potential of $-85.8$ mV was obtained when $\alpha$ is 1.2 ($-95.1$ mV when $\alpha$ is 3), the ratio of values being comparable to the initial and the second peaks, respectively (Kuba et al. 1983; Tokimasa, 1984a). $\alpha$ can be calculated when $V$ and $E_r$ are known. The most representative spike afterhyperpolarization from the previously published data (Tokimasa, 1984a) is 20 mV in amplitude (measured at the second peak) at the resting potential of $-63$ mV. Thus, $V = -20$ mV and $E_r = -63$ mV (therefore, $E'_r = -(63 + 20) = -83$ mV) in Eq (2) and Eq (3) gave 2.6 for $\alpha$ and $-94.5$ mV as the predicted reversal potential (in this case, the measured reversal potential was $-93$ mV (Tokimasa, 1984a)). A value of $\alpha$ of 2.6 implies that the membrane input resistance should decrease from 150 $M\Omega$ to 57.4 $M\Omega$ ($\Delta R_m = -61.7\%$).

**How to estimate $\alpha$ experimentally**

The theoretical calculation of $\alpha$ from Eq (7) has already been described above. $\alpha$ can be estimated experimentally by measuring a decrease in membrane input re-
Ca-INDUCED \( g_k \)-INCREASE

Resistance as follows; \( \alpha = R/R' \), \( (\alpha = R/R') \).
R and R' are the input resistance before and during the afterhyperpolarization, respectively. \( \bar{\alpha} \) and \( R' \) are the maximum value of \( \alpha \) and the minimum value of R' at the peak of the afterhyperpolarization. The estimated \( \bar{\alpha} \) should be 2.6 when R and R' are 150 M\( \Omega \) and 57.4 M\( \Omega \).

It was almost impossible, however, to measure R' precisely at the peak of the afterhyperpolarization in bullfrog sympathetic ganglion cells. This was simply because the membrane time constant (product of membrane input resistance and membrane capacitance) is long (30-120 ms among different cells), whereas the spike

Fig. 6 The estimation of \( \alpha \). A: the spike afterhyperpolarization. Upper trace; hyperpolarizing electrotonic potentials (current-injection; 0.08 nA for 140 ms) were observed during the afterhyperpolarization. A direct spike was evoked at the time indicated by an open circle (resting potential was -72 mV). Lower trace; an saturated electrotonic potential of the same cell (current-injection; 0.08 nA for 740 ms). The membrane input resistance and the time constant were found to 150 M\( \Omega \) and 110 ms, respectively. The input resistance fall during the afterhyperpolarization was clear but one could not quantitate it simply because the membrane time constant was long (110 ms) relative to the duration of the afterhyperpolarization (1060 ms). Broken line shows the afterhyperpolarization without superimposed electrotonic potentials. B: the caffeine-hyperpolarization. Upper trace; evoked hyperpolarization (an antidromic spike indicated by an open circle). Middle trace; rhythmic (spontaneous) hyperpolarization. Hyperpolarizing electrotonic potentials (current-injection; 0.15 nA for 350 ms) were observed during the hyperpolarizations. Input resistance deceased from 200 M\( \Omega \) to 48 M\( \Omega \) at the peak of the hyperpolarizations. Each one division in the bottom trace denotes one second. Caffeine (5 mM) depolarized the membrane from the resting level (-65 mV) to -62 mV.
afterhyperpolarization following a single action potential stays for only tens of ms near its peak (Fig. 6). Post-tetanic hyperpolarization which follows repeated spikes and the caffeine-hyperpolarization also result from an activation of $g_K$ (Kuba, 1980; Kuba and Nishi, 1976; Minota, 1974; Smith et al. 1983; Suzuki and Kusano, 1983). It is, therefore, possible to apply the hypothetical equivalent circuit and Eq (7) for these hyperpolarizations. One could measure $R'$ reliably at the peak hyperpolarizing level of either post-tetanic hyperpolarization or the caffeine-hyperpolarization with taking the advantage of its longer duration (Fig. 6). The particular example in Fig. 6B shows that the caffeine-hyperpolarization was 27 mV in amplitude at the resting potential of -62 mV. The input resistance decreased from 200 M$\Omega$ to 48 M$\Omega$ at its peak. The estimated $\alpha$ (4.17 = 200/48) was in good agreement with the calculated $\alpha$ (4.21 from Eq (7)). This implied that, if one could measure the input resistance decrease with accuracy, a decrease of more than 60% may very well be observed during the spike afterhyperpolarization following a single action potential.

Discussion

The main conclusion from the present study is that one might not have to consider any additional current component other than $g_K$ for any part of the spike afterhyperpolarization. The difference in the reversal potential could be explained if it were altered as a function of $\alpha$ or, more precisely, the ratio $\alpha g_K: g_{Na}$.

Although the equivalent circuit used in the present analysis does predict the difference in the reversal potential, the circuit as it is written in Fig. 4 cannot predict another experimental observation, in that the slower component of the afterhyperpolarization shortens or vanishes with membrane depolarizations (at between -50 and -30 mV) (Fig. 7; see also Kuba et al. 1983) but prolongs with the membrane hyperpolarizations (Kuba et al. 1983; Tokimasa, 1984a). Alternatively, these observations may indicate that time-dependence of $\alpha$ is voltage-dependent. Although this voltage-dependence should be investigated under voltage-clamp conditions (Tokimasa, in preparation), it should be operating in the opposite direction to that expected where one assumes that the voltage-dependence of $\alpha$ reflects the voltage-dependent nature of potassium-channels themselves (Adams et al. 1982; Gorman and Thomas, 1980; Lux et al. 1981; Pallotta.

The most adequate way to estimate the equilibrium potential for potassium ions under current-clamp experiments is to measure the reversal potential of the spike afterhyperpolarization. In this respect, one must take into account that the reversal potential of the slower component of the afterhyperpolarization is closer to $E_K$.

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**References**


