Spontaneous action potential generation due to persistent sodium channel currents in simulated carotid body afferent fibers

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Donnelly DF. Spontaneous action potential generation due to persistent sodium channel currents in simulated carotid body afferent fibers. J Appl Physiol 104: 1394–1401, 2008. First published February 28, 2008; doi:10.1152/japplphysiol.01169.2007.—The mechanism by which action potentials (APs) are generated in afferent nerve fibers in the carotid body is unknown, but it is generally speculated to be a release of an excitatory transmitter and synaptic depolarizing events. However, previous results suggested that Na\(^+\) channels in the afferent nerve fibers play an important role in this process. To better understand the potential mechanism by which Na\(^+\) channels may generate APs, a mathematical model of chemoreceptor nerve fibers that incorporated Hodgkin-Huxley-type Na\(^+\) channels with kinetics of activation and inactivation, as determined previously from recordings of petrosal chemoreceptor neurons, was constructed. While the density of Na\(^+\) channels was kept constant, spontaneous APs arose in nerve terminals as the axon diameter was reduced to that in rat carotid body, AP excitability and pattern were similar to those observed in chemoreceptor recordings: 1) a random pattern at low- and high-frequency discharge rates, 2) a high sensitivity to reductions in extracellular Na\(^+\) concentration, and 3) a variation in excitability that increased with AP generation rate. Taken together, the results suggest that an endogenous process in chemoreceptor nerve terminals may underlie AP generation, a process independent of synaptic depolarizing events.

carotid body chemoreceptor; hypoxia transduction; action potential generation

ACTION POTENTIAL (AP) activity on nerve fibers arising within the carotid body provides information to the brain stem regarding arterial oxygenation status. As arterial PO\(_2\) decreases, AP activity increases. However, the mechanism by which APs arise in the afferent fibers is not well resolved. According to the most widely accepted theory, hypoxia is sensed by carotid body glomus cells, which release one or more excitatory neurotransmitters that bind to classical neurotransmitter receptors located on nerve terminals. This binding is postulated to evoke depolarizing potentials in the nerve fibers, leading to AP generation (29).

However, data supporting this model are limited and inconsistent. Although one recording of cat chemoreceptor nerve terminals appeared to demonstrate depolarization events (29), a similar study in rat nerve terminals demonstrated no events (13). Recently, subthreshold electrical stimulation were used to detect subthreshold synaptic depolarizing events in the carotid body, but no evidence of synaptic depolarizing-like events was found (9, 10). Instead of synaptic depolarizing-like events, the data suggested that fast, small-amplitude events (as would occur with ion channel flicker) were occurring in chemoreceptor nerve terminals and that these events were modulated by O\(_2\) levels. On the basis of the sensitivity of the AP generation process to changes in extracellular Na\(^+\) concentration ([Na\(^+\)]\(_o\)) (10) or Na\(^+\) channel-blocking agents such as lidocaine,riluzole, and phenytoin (10, 16, 17), we proposed that AP generation was due to channel noise associated with Na\(^+\) channel flicker.

To better understand this potential mechanism, a model was developed using Hodgkin-Huxley gating characteristics (34). The parameters for the equations governing activation and inactivation were altered to better reflect the characteristics of Na\(^+\) currents in petrosal chemoreceptor neurons, as determined previously (7, 8). Na\(^+\) channels were modeled as individual channels with discrete Markovian ion kinetics. K\(^+\) and leak currents were modeled with continuous rate equations. The model demonstrates that small nerve fibers, of the size found in rat carotid body, may possess an innate ability to generate APs with several characteristics consistent with those observed in chemoreceptor afferent recordings.

METHODS

The channel characteristics of chemoreceptor afferent neurons were simulated using NEURON version 5.8 (33). Na\(^+\) channels were considered individual channels placed in the center of the soma or axon. K\(^+\) and leak channels were modeled as a distributed process across the surface and computed as continuous rate equations. The integration method was backward Euler, and the integration time step (dt) was 0.0125 or 0.025 ms. Decreasing the integration period had only minor effects on the experimental results.

The voltage change across an element of membrane is given by the following equation: dV/dt = −(1/C)(g\(_{Na}(V−V_l) + gK(V−V_K) − I_{Na})

where C (membrane capacitance per unit surface area), g\(_{Na}\) (leak conductance per unit surface area), and g\(_K\) (K\(^+\) conductance per unit surface area) are distributed processes. V\(_l\) and V\(_K\) are reversal potentials for the leak and K\(^+\) currents. Current from Na\(^+\) channels (I\(_{Na}\)) is a point source on the membrane and summed over all the Na\(^+\) channels to obtain the total I\(_{Na}\).

Passive properties. The passive properties of the cell were based on previous sharp electrode recordings of intact petrosal chemoreceptor neurons (8) and are presented in Table 1. With use of sharp electrode recording, resting potential is approximately −61 mV and input resistance is ∼120 MΩ (8). Somal capacitance of 20 pF was based on previous measurements on dissociated petrosal chemoreceptor neurons (7). If it is assumed that capacitance is 1 μF/cm\(^2\), the cell surface area would be 2.0E-5 cm\(^2\) and normalized conductance would be (1/120 MΩ)/(1/2.0E-5) = 4E-4 S/cm\(^2\) (Table 1).

I\(_{Na}\). Estimates for the model parameters for I\(_{Na}\) were based on the published results of patch-clamp recordings of dissociated petrosal chemoreceptor neurons (7). Chemoreceptor neurons lack TTX-resistant (TTX-R) I\(_{Na}\) which activate 10–20 mV more positive than
Table 1. Parameters used in the simulation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Membrane capacitance</td>
<td>1 µF/cm²</td>
</tr>
<tr>
<td>D</td>
<td>Cylinder diameter</td>
<td>16 µm (soma), 0.5–2 µm (axon)</td>
</tr>
<tr>
<td>L</td>
<td>Cylinder length</td>
<td>40 µm</td>
</tr>
<tr>
<td>Vₙ</td>
<td>Leakage reversal potential</td>
<td>−61 mV</td>
</tr>
<tr>
<td>Gₙ</td>
<td>Leakage conductance</td>
<td>0.4 mS/cm²</td>
</tr>
<tr>
<td>Vₚ</td>
<td>K⁺ reversal potential</td>
<td>−77 mV</td>
</tr>
<tr>
<td>Gₚ</td>
<td>Maximal K⁺ conductance</td>
<td>25 mS/cm²</td>
</tr>
<tr>
<td>[Na⁺]ₙ</td>
<td>Intracellular Na⁺ concentration</td>
<td>10 mM</td>
</tr>
<tr>
<td>[Na⁺]ₑ</td>
<td>Extracellular Na⁺ concentration</td>
<td>140 mM</td>
</tr>
<tr>
<td>Pₙ</td>
<td>Permeability of 1 Na⁺ channel</td>
<td>1E-5 cm/s</td>
</tr>
<tr>
<td>Rₙ</td>
<td>No. of single Na⁺ channels</td>
<td>30 channels/µm²</td>
</tr>
<tr>
<td>Rₚ</td>
<td>Intracellular resistivity</td>
<td>35.2 Ω·cm</td>
</tr>
</tbody>
</table>

TTX-sensitive (TTX-S) Iₙa. Thus the current could be modeled adequately with a single TTX-S-type current. Parameters for activation (V₁/₂ = −23 mV), half-inactivation (Vᵢ/ᵢ = −71 mV), and time constants for inactivation (τₙ = 0.8 ms at −10 mV) were fit to a Hodgkin-Huxley model with the opening of an Na⁺ channel linked to the binding of three activation particles (m) and one inactivation particle (h). As stated by Chow and White (6), the Markov kinetic scheme for the Na⁺ channel is given by

\[
\begin{align*}
3\alpha_{m} & \rightarrow 2\alpha_{m} & \alpha_{m} \\
\beta_{m} & \rightarrow 3\beta_{m} & \beta_{m} \\
\alpha_{n} & \downarrow \downarrow \beta_{n} & \alpha_{n} & \downarrow \downarrow \beta_{n} \\
3\alpha_{h} & \rightarrow 2\alpha_{h} & \alpha_{h} \\
\beta_{h} & \rightarrow 3\beta_{h} & \beta_{h} \\
\end{align*}
\]

with

\[
\begin{align*}
\alpha_{m} &= 0.11133/(V + 40)/(1 - e^{[-0.011(V+40)]}) \\
\beta_{m} &= 12.8e^{-[0.0555(V+65)]} \\
\alpha_{n} &= 1.3[1 + e^{[0.1(V+42)]}] \\
\beta_{n} &= 0.078e^{-[0.051(V+65)]} \\
\end{align*}
\]

The current through an open channel was determined by the Goldman-Hodgkin-Katz current equation

\[
I(Na) = \frac{F^2V}{RT} \left[ [Na^+] - [Na^+]e^{-(FV/RT)} \right] \left[ 1 - e^{-(FV/RT)} \right]
\]

where \( F \) is Faraday’s constant, \( R \) is Ryberg’s constant, \( T \) is absolute temperature, \( P \) is permeability of Na⁺ through a single channel, \( V \) is membrane potential, and \( [Na^+] \) is intracellular Na⁺ concentration.

Na⁺ channels were considered point processes in the membrane and were inserted in the center of the model segment. Stochastic noise is generated from the Na⁺ channels by, for each channel, calculating transition probabilities to neighboring states or staying in the same state after each period, \( dt \). Occasionally, channels would occupy the \( mₙh₁ \) state, corresponding to an open channel, allowing Na⁺ movement.

K⁺ and leak currents. K⁺ currents were modeled as distributed or density processes across the entire membrane and by continuous rate equations with use of the parameters of the original Hodgkin-Huxley model (34).

\[
I_K = g_K*\alpha(Na^+ - V)
\]

where

\[
\begin{align*}
\alpha_n &= 0.01(V + 55)/(1 - e^{[-0.1(V+55)]}) \\
\beta_n &= 0.125e^{-[0.0125(V+65)]} \\
\end{align*}
\]

Leak currents were not voltage dependent and were calculated on the basis of the conductance equation

\[
I_L = g_L(V - V_L)
\]

Comparison of actual data with simulated data. The peak Na⁺ conductance (gₒNa) was calculated from peak \( I_{Na} \) from simulated voltage clamp by use of the conductance equation: \( gₒNa = I_{Na}/(V - VₒNa) \), where \( V \) is the conditioning or test potential and \( VₒNa \) is the estimated reversal potential for Na⁺. Conductance values were normalized to peak values observed during steady-state activation and inactivation and fit to a Boltzmann function, \( gₒNa = I/[1 + exp(−V − VₒNa)/k] \), where \( Vₒ \) is the half-activation or -inactivation potential and \( k \) is a slope factor. For comparison with experimental data, published images of voltage-clamp protocols for \( I_{Na} \) activation and inactivation (7) were digitized (HP ScanJet), and the values were read using an image analysis program (Scion Image). Conductance values were handled as described above.

RESULTS

Na⁺, K⁺, and leak currents were placed initially in a model of the soma, constructed of a 16-µm-diameter 40-µm-long cylinder. In NEURON, axial current flows between segments, but not through membranes, so the “end caps” of the cylinder were not used for determination of area, cell capacitance, or current densities. The surface area of the somal structure was 40 × 16 × 0.002, 000 µm², giving a capacitance of 20 pF. The number of Na⁺ channels in this structure was increased until the somal current replicated the current previously observed in voltage-clamp recordings from 18-day-old petrosal chemoreceptor neurons (7).

Somaal IₒNa. To generate a peak somal current of about −30 nA, the model required placement of 36, 000 channels in the somal structure (Fig. 1). This is higher than the minimum required (15, 000) based on the single-channel current, which was −2 pA because of the failure of some channels to open after depolarization and asynchronous openings of channels. The estimated number of channels compares favorably with the number of saxitoxin-binding sites on neuroblastoma cells, which has been estimated at 50, 000 (55). The half-inactivation potential was approximately −65 mV (Fig. 1). The values are comparable to that obtained in patch-clamp measurements from P18 dissociated petrosal neurons with projections to the carotid body (7). The noninactivating portion of the current was −1% of peak current, which is similar to that reported for the noninactivating portion of TTX-S isoforms in expression systems (51).

Axonal IₒNa. Placement of the same Na⁺ channels at the same density found at the soma in a model axon of differing diameters resulted in the generation of voltage noise due to the episodic openings of individual Na⁺ channels (Fig. 2). The magnitude of the noise increased as the axonal diameter decreased. At 0.1 µm diameter, the noise caused by these channel transitions evoked APs, and the rate increased as the axonal diameter was further decreased (Fig. 2). This critical diameter (<1 µm) corresponds to the size of unmyelinated chemore-
ceptor axons in the rat carotid body (42, 43). AP height was variable because of the small number of channels in the membrane and the starting conditions, which placed more than half the Na
\(^{+}\)/H
\(^{+}\) channels in the inactive state.

The depolarization events in the axon were caused by the opening of a small number of Na
\(^{+}\)/H
\(^{+}\) channels, approximately 3–4 of the total of 3,000 channels in the axonal model (Fig. 3). The axon was 0.75 \(\mu\)m diameter and 40 \(\mu\)m long. The open channel could potentially be caused by a transition from the third closed state (m2h1) to the open state (m3h1) or by a transition from the fourth inactive state (m3h0) to the open state. However, because of the variability in the number of channels in m2h1 and m3h0 and the low number of channels that transitioned to the open state, it was impossible to identify the source of the open channel.

**Interspike interval distribution.** Interspike intervals were calculated using axons with a diameter of 0.75 \(\mu\)m, which is the average diameter of axons in rat carotid bodies (42, 43). Under baseline and high rate conditions induced by injection of a 0.003-nA current, the discharge pattern appeared random and the interspike interval distributions were well fit to single-exponential functions (Fig. 4). In addition to the overall distribution of intervals, the interspike intervals had other characteristics of a random process. The square root of the mean interval was approximately the same as the standard deviation (SD) of the intervals (Fig. 4). At the slower spiking rate, the mean period was 301 ms (SD 305). For the faster rate, the mean period was 47.7 ms (SD 43.8) (Fig. 4).

**Sensitivity to reduction in [Na
\(^{+}\)].** Previous experimental results from this laboratory (10) and others (21) demonstrated a high sensitivity of the chemoreceptor spike generation process to reductions in [Na
\(^{+}\)]. In our model, a reduction in [Na
\(^{+}\)]\(_o\) would reduce the current through an open Na
\(^{+}\)/H
\(^{+}\) channel, the flow of which was approximated by the Goldman-Hodgkin-Katz constant-field equation. The spontaneous AP generation rate was highly sensitive to reductions in [Na
\(^{+}\)]\(_o\) (Fig. 5), with an \(\sim 70\%\) reduction in frequency following a 10% reduction in [Na
\(^{+}\)]\(_o\).

**Variability in the threshold for spike generation.** The ability of an electrical stimulus to evoke an AP was examined by placement of a current source within the axon and application of a short (0.1-ms) current pulse. Each current was applied 100 times, and the probability of successfully evoking an AP was graphed against the magnitude of the injected current. The probability of successfully initiating an AP increased as the stimulus current was increased. The relationship was analyzed using a modification of the model developed by Gallego et al. (21) in which the probability of spike generation was modeled as the probability of exceeding an energy barrier. The proba-

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**Fig. 1.** Simulated voltage-clamp currents showing activation (A) and steady-state inactivation (B) characteristics when placed in a soma at a density of 30 channels/\(\mu\)m\(^2\) of membrane surface. Magnitude of transient Na
\(^{+}\) current (I\(_{\text{NaT}}\); peak current \(\sim 30\) nA) approximates that previously recorded on dissociated petrosal neurons with projection to the carotid body (7). Noninactivating portion of I\(_{\text{NaT}}\) is postulated to account for the persistent sodium current (I\(_{\text{NaP}}\), “1% of peak”). C: voltage dependence of steady-state activation (○) and inactivation (□) for simulated Na
\(^{+}\) channels. D: voltage dependence of steady-state activation (○) and inactivation (□) of Na
\(^{+}\) currents from published data on chemoreceptor neurons (7). Half-activation potentials for the model and digitized data were \(-22\) and \(-20.5\) mV, respectively. Half-inactivation potentials were \(-69\) and \(-70\) mV, respectively. Integration time constant (dt) was 0.0125 ms.

**Fig. 2.** Spontaneous action potential (AP) generation from modeled axons during reductions in fiber diameter. A: spontaneous AP activity was present at \(\leq 1\) \(\mu\)m diameter, and AP frequency increased as diameter decreased. Channel density remained constant at 30/\(\mu\)m\(^2\). B: AP frequency vs. channel number for 0.75-\(\mu\)m-diameter axon; dt = 0.0125 ms.
bility distribution across the energy barrier is as follows: 
\[ p(\text{AP})/p(\text{noAP}) = C e^{-U/kT} \]
where \( C \) is a constant, \( U \) is an activation energy, \( k \) is Boltzmann’s constant, and \( T \) is absolute temperature. The probability of an AP is as follows: 
\[ p(\text{AP})/p(\text{noAP}) = 1/[1 + e^{(U/kT)}] \]
where \( x_0 \) is the stimulus current that generates an AP half of the time and \( d \) is a slope factor.

The relationship between current magnitude and probability of success could be well fit to a Boltzmann function (Fig. 6). A reduction in spontaneous spike probability caused by a hyperpolarization of the axon (due to a sustained current of \(-0.003 \text{nA}\)) raised the current required to obtain a 50% success of AP generation by 18% and increased the slope factor of the Boltzmann function by 36.8% (Fig. 6). The magnitude of bias current was designed to induce approximately the same change in discharge frequency observed in a previous study when conditions were switched from normoxia to hyperoxia (9).

![Fig. 3](image_url)

**Fig. 3.** Time relationship between subthreshold voltage changes, number of open Na\(^+\) channels, and 2 states that may transition to the open state, \( m_3h_1 \) and \( m_3h_0 \). Ordinate values represent the number of channels in the specified state. Depolarization events were driven by a small number of open Na\(^+\) channels (arrows), but whether few open channels were produced from transitions from \( m_3h_1 \) to open or \( m_3h_0 \) to open was unresolved. *, AP occurrence times; \( dt = 0.025 \text{ ms} \).

![Fig. 4](image_url)

**Fig. 4.** Interspike interval distribution of APs generated from a 0.75-μm-diameter modeled axon. Interspike interval distribution was exponential (dotted line) under high rate (A) and low rate (B) states. Standard deviation (SD) also approximated the mean for high rate [47.7 ms (SD = 43.8)] and low rate [301 ms (SD 305)] states. Inset: 10-s polygraphic trace of simulated membrane potential; \( dt = 0.025 \text{ ms} \).

![Fig. 5](image_url)

**Fig. 5.** Spike generation rate is dependent on extracellular Na\(^+\) concentration ([Na\(^+\)]\text{o}). Reductions in [Na\(^+\)]\text{o} reduce current through open Na\(^+\) channels on the basis of the constant-field equation. Predicted effect is a reduction in spontaneous AP frequency compared with previously published observations of chemoreceptor activity during isosmotic reductions in Na\(^+\) (10).

![Fig. 6](image_url)

**Fig. 6.** Variability of axonal excitability as assayed by AP generation during application of a brief (0.1-ms) stimulus current. Each point represents success rate of AP generation following application of a 0.1-ms stimulus current pulse and different intensities. Data points are fit to a Boltzmann distribution: 
\[ p(\text{success}) = 1/[1 + e^{(x_0/x)dt}] \]
where \( x_0 \) is the current that evokes an AP half of the time and \( dx \) is a slope factor. A lower spiking rate (○) caused by application of a continuous bias current of \(-3 \text{ pA}\) increased \( x_0 \) by 18% to 0.104 nA and increased slope factor by 37% to 0.012.
A change in the rate constants governing transition between closed and inactive states may increase the amount of noninactivating current while leaving the amount of inactivating (transient) current the same. For instance, a doubling of the cofactor for $b_0$ from 0.07 to 0.14 causes no change in the simulated transient current evoked by a depolarization from $-100$ to $-10$ mV (Fig. 7) but approximately doubles the amount of noninactivating current from 1% to 2% of peak. This has a large effect on spontaneous AP activity of simulated small axons (Fig. 7), suggesting that processes that modulate the transition between states of the Na$^+$ channel may (potentially) change the rate of AP generation.

**DISCUSSION**

The principal conclusion from the present work is that Na$^+$ channels in small nerve fibers may generate APs directly depending on the physical characteristics of the nerve terminals and the density/biophysical characteristics of the Na$^+$ channels. When parameters developed from somal recordings of chemoreceptor neurons and morphological measurement of chemoreceptor nerve fibers are applied, the model predicts that spontaneous APs may be generated in these fibers as a result of depolarization events caused by episodic openings of Na$^+$ channels.

These depolarization events are due to overlap, around the resting potential, of the steady-state inactivation and activation processes of the Na$^+$ channel ("window current"). From experimental work using tuberomammillary neurons (52) and medullary neurons (50), it can be concluded that all persistent sodium current ($I_{NaP}$) in these neurons may be accounted for by this process. However, other experimental work suggests that $I_{NaP}$ may be due to different Na$^+$ channels or different gating modes of the Na$^+$ channel based on differences in single-channel conductance between the transient and persistent currents (40, 41) and, thus, may require modeling as a separate channel entity (31). For the present work, $I_{NaP}$ in chemoreceptor neurons is assumed to be generated solely by a window current but may necessitate modification once single-channel data are available from these chemoreceptor neurons.

The present modeling result predicting spontaneous AP generation in small fibers is broadly consistent with that reported in a modeling study by Chow and White (6), who applied a Hodgkin-Huxley model of Na$^+$ and K$^+$ channels, at fixed density, to small nerve fibers. They observed that as the nerve diameter decreased, the tendency to generate APs increased. Although the present study reaches the same conclusion, the modeling parameters are different in several respects. 1) The membrane noise generated in the model of Chow and White, which gave rise to APs, was due to Na$^+$ and K$^+$ channels, and the source was not discriminated or apportioned. Since chemoreceptor activity is relatively insensitive to K$^+$ channel-blocking agents (5, 12, 39), K$^+$ channels in this study were not considered on an individual channel basis and, hence, were considered noiseless. 2) Chow and White fixed the Na$^+$ channel density at 60 channels/$\mu$m$^2$, which was double that used in the present study. 3) Voltage dependence of state transitions used in the present study was based on previous recordings of chemoreceptor neurons and was different from that used in the model of Chow and White.

The higher excitability of small nerve fibers at fixed Na$^+$ channel density is due to the reduced spatial filtering of the small nerve fibers. The smaller-diameter fibers possess less capacitance per unit length and a smaller wavelength due to a higher axial resistivity. Thus, in small axons, the fortuitous, simultaneous opening of one to three Na$^+$ channels may lead to a depolarization event, which reaches threshold. Although this may occur potentially anywhere along the axon, the area near the nerve terminal would have less spatial filtering, since the axon extends in only one direction, rather than two directions, from the depolarizing current. Of relevance to chemoreceptor axons, this model of AP generation predicts several established characteristics of chemoreceptor afferent activity, including spike distribution, sensitivity to reductions in [Na$^+$]$\theta$, and variations in axonal excitability.

It has long been established that chemoreceptor spike generation occurs in a random pattern, essentially similar to that of a radioactive source, and maintains this pattern at low and high levels of stimulation (2, 11, 14). Some deviation is reported in some species (goats and birds) because of the generation of "doublets" (45) or modulation by the respiratory and cardiac cycles, but once these factors are eliminated, the discharge pattern is random (22, 23, 44, 46). The discharge pattern produced by the current model possesses similar characteristics. The interspike interval distribution is exponential under low and high levels of stimulation, and the mean of the interspike period approximates the SD of the interspike periods (Fig. 4). The maintenance of a random pattern under high levels of stimulation is not shared by most sensory systems. For instance, lobster stretch receptors (15) and frog muscle spindles (38) discharge with irregular patterns at low levels of...
stretch but highly regular patterns at higher levels of stimulation.

The predicted spike generation rate is also highly sensitive to reductions in $[\text{Na}^+]_o$, predicting an $\sim 70\%$ decrease in AP frequency for a $10\%$ decrease in $[\text{Na}^+]_o$ (Fig. 5). Previously, a decrease in chemoreceptor activity was observed after an osmotic decrease in $[\text{Na}^+]_o$, but the magnitude of decrease was less than that predicted by the present model (10, 21). A $\sim 30\%$ decrease in spontaneous AP rate for a $10\%$ decrease in $[\text{Na}^+]_o$ was observed (10). The higher sensitivity of the model may be potentially explained by several factors. Experimental reductions in $[\text{Na}^+]_o$ may not have equilibrated fully with tissue Na$^+$ levels, resulting in an overestimation of the actual Na$^+$ reduction. Also, the present model utilizes the constant field equation for predicting current through an open Na$^+$ channel. However, channels may present positive and negative cooperativity among multiple ions within the channel pore (32). This may account for a greater or lesser conductance change following a given change in ion concentration. Since ionic current changes caused by changes in $[\text{Na}^+]_o$ have not been investigated in chemoreceptor neurons, the constant field equation should be viewed as providing only an approximation of the actual current change. Alternatively, a reduction in $[\text{Na}^+]_o$ would reduce the electrochemical gradient for Na$^+$, thus reducing the transport ability of Na$^+$-driven pumps, but the effect is likely to be minor. A $20\%$ reduction in $[\text{Na}^+]_o$, from 140 to 112 mM, would lower $V_{\text{Na}}$ from 66 to 60.8 mV. If the membrane potentials were $-60$ mV, then the reduction in potential energy from the translocation of an Na$^+$ ion would be only $4.1\%$.

Another prediction of the model that is consistent with an observation in chemoreceptors is the change in variability of excitability with depolarization or hyperpolarization. Previously, we applied graded electrical stimuli within the carotid body to determine the relationship between stimulus intensity and probability of evoking an orthodromic AP (9). This relationship could be well fit to a Boltzmann function, characterized by a stimulus value $x_0$, which yields a $50\%$ probability of successfully evoking an AP, and a slope factor, which indicates the sensitivity to changes in stimulus strength. A decrease in spontaneous AP rate, caused by increasing O$_2$, increased slightly $x_0$ and also increased the slope factor (9). Similar results were found in the present model. The relationship between stimulus intensity and AP generation probability was well fit to the Boltzmann function, and hyperpolarization caused an increase in slope factor (Fig. 6). This result is consistent with that obtained on nodal Na$^+$ channels, where changes in slope factor with hyperpolarization or depolarization reflect primarily changes in $I_{\text{NaP}}$ secondary to membrane potential changes (25).

If AP generation in chemoreceptor nerve fibers is caused by $I_{\text{NaP}}$, a result consistent with the present model, then the generation rate should be sensitive to drugs that reduce $I_{\text{NaP}}$. Previously, we demonstrated that riluzole and phenytoin, both of which stabilize the inactive state of the Na$^+$ channel and reduce $I_{\text{NaP}}$, greatly reduce spontaneous AP generation rate in isolated chemoreceptors (16, 17). When given in vivo, the same drugs nearly ablate the respiratory response to acute hypoxia but have little effect on the respiratory response to acute hypercapnia (16, 17). AP generation through an endogenous process is also broadly consistent with an anatomic and electrophysiological assessment of nerve-glomus cell interaction. Electron-microscopic examination shows a weak relationship between fusion profiles of glomus cell secretory granules and the location of nerve fibers (24, 54). Thus SDPs produced by these fusion events (if SDPs are actually produced) would be slowed and reduced in amplitude as a result of spatial diffusion. In a recent electrophysiological examination in the rat of the nerve-glomus cell interaction, Eyzaguirre (13) failed to detect any evidence of SDP-like coupling between glomus cells and nerve endings, a result that Eyzaguirre characterized as “puzzling,” but one consistent with an AP generation process as described here.

Although the present results indicate that APs may be generated in small nerve fibers as a result of Na$^+$ channel fluctuations, the model provides no information on how the process is modulated. A number of purported excitatory transmitters, including acetylcholine, ATP, dopamine, substance P, and 5-HT, are synthesized and released from carotid body cells (56). Any or all of these substances may be released during hypoxia and cause nerve terminal depolarization and enhanced AP generation. Alternatively, transduction of hypoxia could, potentially, occur within the nerve terminal itself. In several O$_2$-sensitive cells, including hippocampal neurons, hypothalamic neurons, and heart muscle, the biophysical characteristics of Na$^+$ channels are altered by hypoxia (20, 26–28, 35–37). Hypoxia shifts the voltage of activation in the hyperpolarized direction, resulting in an increase in $I_{\text{NaP}}$ around the resting potential. Even a small increase in $I_{\text{NaP}}$ may have a large effect on the AP generation rate (Fig. 7) and, thus, indicates the potential to contribute significantly to the response of the carotid body to hypoxia.

Despite some success in predicting the excitability characteristics of nerve terminals, the present model should, at best, be considered rudimentary. On the basis of immunological studies, petrosal chemoreceptor neurons express at least seven types of K$^+$ channels (1). An electrophysiological characterization of the petrosal chemoreceptor K$^+$ currents is lacking, but it is likely that default Hodgkin-Huxley values do not represent a good fit. Similarly, no data are available on any Ca$^{2+}$ currents in these cells. In addition, our model also assumes a similar complement of ion channels between the soma and nerve terminals. However, changes in channel density and isoforms are observed between the neuronal soma and other axonal areas. For instance, the density of Na$^+$ channels is greater in the initial segment than the cell body (4), and TTX-R channels are localized at the soma and nerve terminals of corneal axons but do not appear to be present along the conducting portion of the axon (3). Thus the present model may be improved considerably once Ca$^{2+}$ and K$^+$ currents of petrosal chemoreceptor neurons are characterized and after the channel distribution is better defined.

Although the present model suggests that noise generated from Na$^+$ channels may underlie generation of chemoreceptor APs, this should not be viewed in exclusion of other possible mechanisms. Work in other laboratories has suggested critical roles for substance P (47–49), acetylcholine (18, 19, 30, 58), or acetylcholine combined with ATP (53, 57) in hypoxia transduction and, perhaps, spike generation. If the nature of the chemical coupling was discerned or, at least, postulated, it would allow defined models to be developed and model predictions to be tested. For instance, a purported transmitter may
cause 1) a sustained nerve depolarization, 2) an SDP that exceeds threshold with a safety factor (e.g., neuromuscular transmission), or 3) subthreshold SDPs, which require summation to reach threshold. Each postulate would yield a different model with a defined relationship between transmitter release and AP activity that could be experimentally verified or rejected. However, transmission characteristics have not been postulated or defined for any purported transmitter.

In summary, the simulated placement of Na⁺ channels, the density and electrical characteristics of which are similar to those in the soma of chemoreceptor afferent neurons, in fine nerve terminals produce APs with some characteristics similar to those observed in chemoreceptor recordings, including a random discharge pattern during stimulated and unstimulated conditions. However, transmission characteristics have not been postulated or defined for any purported transmitter.

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**REFERENCES**