Experimental and Modeling Study of the Excitability of Carotid Sinus Baroreceptors

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In this study we examined the effects of blockade of a transient K⁺ current with 4-aminopyridine (4-AP) on the static stimulus-response relation of myelinated carotid sinus baroreceptors (n=8), using a vascularity isolated sinus preparation in sodium thiopental–anesthetized dogs. In one class of baroreceptors (type I), which did not fire spontaneously below the pressure threshold (Pth), 4-AP (10⁻⁶ to 10⁻⁴ M) decreased Pth in a dose-dependent manner and transformed the stimulus-response relation from a discontinuous, hyperbolic shape to a sigmoidal, continuous curve. After exposure to 10⁻⁴ M of 4-AP, baroreceptors were spontaneously active below Pth. These effects of 4-AP were more pronounced in baroreceptors with a high control Pth and were independent of enhanced neurotransmitter release or changes in carotid sinus distensibility. In contrast, 4-AP had relatively little effect on type II baroreceptors, which under control conditions are characterized by a continuous, sigmoidal stimulus-response curve. We believe that these effects of 4-AP on baroreceptor discharge were mediated by blockade of a transient K⁺ conductance that was present at the receptor spike-initiation zone. This hypothesis was examined using a mathematical model based on the Hodgkin-Huxley axon, but modified to include the transient K⁺ conductance. The modeling results showed that the minimum current necessary to elicit action potential firing is an extremely sensitive function of the magnitude of this K⁺ conductance, supporting our experimental results obtained with 4-AP. Our findings suggest that a transient K⁺ conductance might play a role in the determination of Pth and that differences between type I and II receptors could be the result of differences in the effectiveness of this conductance in controlling spike-initiation zone excitability. (Circulation Research 1990;66:1510–1525)

In our accompanying article in this journal,¹ we described two types of baroreceptors in the carotid sinus of the dog: 1) type I baroreceptors, which responded to a slow ramp increase in pressure with a sudden onset of discharge at the minimum threshold pressure (Pth) and an initially steep increase in firing rate thereafter to saturation pressure, and 2) type II baroreceptors, which fired spontaneously below the Pth and gradually increased their firing frequency above this threshold. It has been suggested that the electrophysiological properties of the baroreceptor membrane could be involved in generating the discontinuity in the stimulus-response curves of type I baroreceptors.²³

Unfortunately, the mechanosensitive nerve endings are too small to permit direct studies of electrical events during the mechanoelectrical transduction process. Studies in invertebrate mechanoreceptors, thought to be functionally analogous to arterial baroreceptors,² have shown that mechanical deformation of the receptor membrane leads to a depolarizing generator current that spreads electrotonically to a low-threshold site on the axon hillock or initial segment.³⁴ If the generator current exceeds the firing threshold of this Na⁺ channel–rich region, usually called the spike-initiation zone (SIZ), action potentials will be generated. Mechanoreceptors can vary their firing frequency over a wide range in response to excitatory stimuli, and under steady-state conditions, the relation between stimulus strength and firing frequency is linear.¹³⁻⁵ It is believed that these spike-encoding properties are determined by a complex balance of K⁺ currents activated at sub-threshold and near-threshold membrane voltages.⁶⁻⁷ A transient K⁺ current, the A current (IA), is one of these currents. It has been shown in crustacean axons⁸ and sympathetic neurons⁹ that IA is essential for repetitive firing in response to a depolarizing stimulus. In addition, IA functions to reduce the responsiveness of mammalian neurons to excitatory stimuli and can thereby determine the firing thresh-
old of neurons. The role of $I_h$ has been studied using the convulsant drug 4-aminopyridine (4-AP), which selectively blocks $I_h$ in most neurons when applied externally in the low millimolar range. At higher concentrations of 4-AP, an additional blockade of the delayed outward $K^+$ current ($I_K$) is usually found.

Although $I_h$ is present in nearly all mammalian neurons, it is not known whether this current is involved in the determination of the excitability of arterial baroreceptors. In this study we examined the effects of 4-AP ($10^{-5}$ to $10^{-4}$ M) on the response of single carotid sinus baroreceptors to a slow ramp increase in intrasinus pressure using a vascularly isolated sinus preparation in the anesthetized dog. To eliminate the possibility that the effects of 4-AP are dependent on the associated release of a sympathetic neurotransmitter, the baroreceptor response to 4-AP exposure was also examined in the presence of the $\alpha$-blocker phentolamine.

Since the electrophysiological properties of arterial baroreceptors, and therefore the blocking actions of 4-AP, cannot be examined directly, we used a mathematical model of the baroreceptor SIZ based on numerical solutions of a modified Hodgkin-Huxley (H-H) model for action potential generation in an axon to examine the possible roles played by $I_h$ and $I_K$ in regulation of baroreceptor excitability. The original H-H model was modified to include $I_h$ as described by Connor et al. The amplitudes of $I_h$ or $I_K$ in the model were reduced to simulate the blocking actions of 4-AP and to study the effects of these manipulations on the excitability of the model axon. Mathematical modeling of the spike-encoding step in arterial baroreceptors provided a theoretical framework for the interpretation of the differences in baroreceptor excitability and the effects of 4-AP.

Materials and Methods

Single-Fiber Carotid Sinus Baroreceptor Recordings

Eight mongrel dogs (15–25 kg) were anesthetized with sodium thiopental (25 mg/kg) and maintained on a thiopental sodium infusion of 10 mg/kg/hr, which has previously been found to maintain a stable level of anesthesia. Each animal was intubated and ventilated with 100% $O_2$. Blood gases and $pH$ were monitored and maintained within the normal ranges by adjustments of ventilation and infusion of sodium bicarbonate. The left carotid sinus was vascularly isolated using a technique explained previously. The left carotid sinus nerve was identified by the characteristic baroreceptor afferent pattern synchronous with changes in carotid sinus pressure (CSP). The nerve was then sectioned near its junction with the glossopharyngeal nerve, desheathed, and dissected into smaller bundles until a preparation was obtained that contained only one active fiber that fired in response to changes in CSP. Single-fiber nerve activity was then recorded as described in our accompanying paper in this journal.

Before examination of baroreceptor stimulus-response relations, the carotid sinus was perfused for a minimum of 1 hour at a constant pulsatile pressure with a mean of 110 mm Hg. Control baroreceptor responses to slow increases in CSP were then obtained as described earlier. The carotid sinus was then perfused for 3 minutes with perfusate containing $10^{-3}$ M of 4-AP at the servocontrolled mean CSP of 110 mm Hg, and then the pressure ramps were repeated. This sequence was repeated for 5$ \times 10^{-5}$ M and $10^{-4}$ M of 4-AP. In four of the eight experiments, the sinus was exposed to 4-AP in combination with the $\alpha$-blocker phentolamine ($10^{-6}$ M). Phentolamine has been shown to block the effects of 4-AP on smooth muscle and to block the effects of catecholamines on baroreceptor discharge.

Reversibility of the effects of 4-AP was tested in three fibers. Perfusate containing 4-AP was washed out, and new control perfusate was circulated for 1 hour. End-control responses were then obtained and compared with the initial ramps. In addition, the effects of tetraethylammonium (TEA), a known blocker of $I_K$, were tested on one baroreceptor. After the control ramps, the sinus was perfused with $10^{-3}$, $5 \times 10^{-3}$, and $10^{-2}$ M TEA, followed by $10^{-4}$ M of 4-AP in the presence of the highest dose of TEA.

At the end of each experiment, the conduction velocity of the fibers studied was obtained as described in the accompanying paper. CSP-baroreceptor discharge relations were constructed for each baroreceptor fiber before exposure for control conditions and after exposure to different concentrations of 4-AP, phentolamine, and/or TEA. Data sampling and computer analysis were performed as described previously. Typically, the static response of carotid sinus baroreceptors with myelinated afferents in dogs is characterized by a distinct discontinuity at $P_{th}$ at which these baroreceptors suddenly start firing at a rate that is called the threshold frequency ($F_{th}$). Baroreceptor impulse discharge at suprathreshold pressures is characterized by an initial linear increase in frequency with increasing CSP followed by a gradual plateau at high CSP until a saturation frequency ($F_{sat}$) is reached. In our experience, this type of discontinuous, hyperbolic pressure-frequency curve, which has been designated as a type I response curve, can be described as

$$F = F_{sat} + (F_{th} - F_{sat}) e^{-k(P-P_{th})}$$

where $F$ is the static baroreceptor firing frequency (spikes per second), $k$ is a slope parameter, and $P$ is equal to CSP. The initial slope, used as an index of baroreceptor sensitivity, is given by

Slope = $k (F_{sat} - F_{th})$

Baroreceptor sensitivity was expressed as spikes per second per millimeter mercury. In some cases, addition of 4-AP to the sinus perfusate resulted in a change in shape of the pressure-frequency relation from hyperbolic to sigmoidal. This type of curve, designated a type II baroreceptor response curve,
was described using the logistic function given here as

\[ F = F_{th} + \left( F_{sat} - F_{th} \right) \left/ \left( 1 + e^{(P_{1/2} - P)} \right) \right. \]

(2)

where \( P_{1/2} \) is the CSP at which \( F \) is half maximal. Baroreceptor sensitivity under these conditions was expressed as the slope of the linear portion of the curve at CSP=\( P_{1/2} \), or

Slope \( = k \left( F_{sat} - F_{th} \right) / 4 \)

\( P_{th} \) for this type of pressure-frequency curve was calculated from

\[ P_{th} = P_{1/2} - (2.634/k) \]

where \( P_{th} \) equals the intrasinus pressure at which firing frequency starts to increase from the spontaneous firing rate in response to ramp pressure.\(^1\) Equations 1 and 2 were curve-fitted to the experimental data using nonlinear least-squares regression techniques.\(^24\) Differences in the calculated curve parameters \( P_{th}, F_{th}, \) slope, and \( F_{sat} \) under control conditions and after baroreceptor exposure to 4-AP were tested using a two-way analysis of variance and Duncan’s multiple-range test, with significance set at \( p < 0.05 \).

**Mathematical Modeling of the Baroreceptor Axon**

Our model uses formulations for gating of ionic channels described originally by Hodgkin and Huxley.\(^18\) The equations for the calculations of the ionic conductances, gating parameters, and analytical expressions for the rate constants are given in the “Appendix.” The spike-encoding properties of the model axon in response to a steady injected depolarizing current were investigated to simulate the encoding of a steady generator current by the baroreceptor SIZ. The original equations, developed for the squid axon at 6°C,\(^18\) were modified to enable repetitive firing of the baroreceptor at a temperature of 37°C. The resulting discharge pattern is characterized by 1) a sudden onset of firing when the injected current exceeded the threshold (firing threshold), 2) a large frequency-modulation range, and 3) a linear relation between current and firing frequency. The first modification involved addition of a transient \( K^+ \) conductance to the H-H axon as suggested by Connor et al.\(^19\) In response to a constant injected stimulating current \( I(t) \), the net current across the model axonal membrane \( (I_n) \) is given by the sum of the capacitative current \( (I_c) \), the ionic currents for \( Na^+ \) \( (I_{Na}) \) and \( K^+ \) \( (I_K \) and \( I_{A}) \), and the leakage current \( (I_L) \):

\[ I(t) = I_n = I_c + I_{Na} + I_K + I_A + I_L \]

The amplitude of the individual ionic currents is given by the product of the membrane conductance for each ion and the electromotive driving force (see “Appendix”). The total membrane current was calculated at the point of injection of current, ignoring propagation of action potentials; that is, membrane potential is considered to change simultaneously at all points along the axon, a condition that is commonly referred to as “space clamp.” The second modification involved the generation of a more pronounced spike undershoot or after-hyperpolarization (AHP) than the original H-H model, which has been found to be a characteristic feature of transducer-encoder neurons.\(^6\) To limit the computational complexities of the model, we adopted the relatively simple method developed by Shapiro and Lenherr,\(^25\) which generates a larger AHP by slowing the rate of repolarization through an increase in the time constant \( \tau_r \) of the activation parameter \( n \) of \( I_K \) in the subthreshold membrane voltage range. \( \tau_r \) determines the rate at which \( I_K \) develops in response to a membrane depolarization, and in our study \( \tau_r \) was multiplied by a voltage-dependent function \( (\gamma) \), which increases the ratio of the value of \( \tau_r \) at the resting membrane potential versus the value of \( \tau_r \) at 0 mV compared with the H-H model.\(^25\) In our study, the standard value of the ratio 1/\( \gamma = \gamma_0 \) at a membrane potential of −60 mV was set at 2, such that at this potential \( \tau_r^* = \gamma_0 \cdot \tau_r = 2 \cdot \tau_r \) (for a more detailed description of \( \gamma \) see “Appendix”). Other (minor) modifications of the H-H model involved a shift toward more depolarized levels of the membrane potential at which \( I_{Na} \) activates and inactivates to ensure full-size action potentials during the entire spike train and a weakened \( I_K \) in the subthreshold membrane voltage range, which is a characteristic feature of repetitively firing neurons.\(^3,6\)

The firing characteristics and excitability of the model baroreceptor SIZ were investigated by stepping up the injected depolarizing current level from zero in steps of increasing amplitude until the axon started to fire. Once the threshold current and corresponding threshold firing frequency were determined, current amplitude was increased further until a current amplitude of 5 \( \mu A/cm^2 \) was reached. From the amplitude of injected current and the corresponding firing frequency we determined the steady-state current-frequency relation or stimulus-response curve of the model axon. The blocking effect of 4-AP on the transient \( K^+ \) conductance\(^20\) was simulated in our model by decreasing the value of \( I_K \) through a decrease in the maximum transient \( K^+ \) conductance \( (g_K) \) and examining the effects of this manipulation on the threshold current and slope of the current-frequency relation. Similarly, reductions in the value of the maximum delayed outward \( K^+ \) conductance \( (g_{K}) \) or the duration and size of the AHP (through changes in \( \gamma_0 \)) were used to simulate possible nonselective blocking effects of 4-AP on \( I_K \) and to study the effects of changes in these parameters on the current-frequency relation.

The H-H differential equations, described in the “Appendix,” were solved by numerical integration by computer (model 310, Hewlett-Packard, Palo Alto, California). Adams’ method was used to perform the integrations, and a second-order predictor and a third-order corrector formula were used to construct the interpolating polynomial for the derivative function based on an algorithm described by Plant.\(^26\)
Results

Experimental Results

Addition of 4-AP to the carotid sinus perfusate resulted in profound changes in the relation between ramp CSP and carotid sinus baroreceptor firing frequency as illustrated in Figure 1. The control baroreceptor stimulus-response curve (top panel) was characterized by the absence of firing below a pressure of 85 mm Hg, followed by a sudden jump in frequency at the $P_{th}$ to $F_{th}$ of 17 spikes/sec. At sinus pressures above $P_{th}$ the firing frequency was a nonlinear, saturating function of ramp CSP. A conduction velocity of 3.8 m/sec indicated that this baroreceptor was connected to a myelinated fiber. Eight myelinated fibers had similar discontinuous, hyperbolic type I stimulus-response curves under control conditions, with mean values for control $F_{th}$, $P_{th}$, slope, and $F_{sat}$ given in Table 1. In the representative example shown in Figure 1, the lowest dose of 4-AP produced a shift to the left of $P_{th}$ to 70 mm Hg and a lowering of $F_{th}$ to 12 spikes/sec. Increasing the dose of 4-AP to $5 \times 10^{-5}$ M further decreased $P_{th}$ to 49 mm Hg and $F_{th}$ to 6 spikes/sec. At the highest dose of 4-AP, this baroreceptor became spontaneously active with a firing threshold (point of onset of discharge, not pressure related) below 20 mm Hg and a $P_{th}$ (pressure at which baroreceptor discharge becomes pressure sensitive) of 50 mm Hg and $F_{th}$ of 5 spikes/sec. The shape of the pressure-frequency curve was gradually changed from hyperbolic to sigmoidal with increasing concentrations of 4-AP, and the discontinuity at $P_{th}$ was absent with $10^{-4}$ M of 4-AP (Figure 1).
Table 1. Values for Threshold Pressure, Threshold and Saturation Frequencies, and Slopes for Type I Baroreceptors in Response to Exposure to 4-Aminopyridine

<table>
<thead>
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<th>Control (n=8)</th>
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<tr>
<td></td>
<td>10^-5 M (n=8)</td>
<td>5×10^-5 M (n=8)</td>
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<tr>
<td>Threshold pressure (mm Hg)</td>
<td>81.3±13.4</td>
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<td></td>
<td></td>
<td>41.5±8.6†</td>
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<td>Threshold frequency (spikes/sec)</td>
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<td>12.6±4.1</td>
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<td>Saturation frequency (spikes/sec)</td>
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<td></td>
<td></td>
<td>37.9±5.8</td>
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<tr>
<td>Slope (spikes/sec/mm Hg)</td>
<td>0.602±0.153</td>
<td>0.499±0.136</td>
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<td></td>
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<td>0.513±0.147</td>
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Values are mean±SEM.

*p<0.05 vs. control.

†p<0.05 vs. control and 10^-4 M 4-aminopyridine.

The effects of 4-AP on type I carotid sinus baroreceptor stimulus-response curves were unaffected by prior α-adrenergic blockade by phentolamine, as illustrated in Figure 3 for the same baroreceptor as shown in Figure 1. As in our previous studies, phentolamine by itself had no significant effects on control stimulus-response curves. Addition of 4-AP (10^-4 M) to a perfusate containing 10^-6 M phentolamine resulted in a decrease in Pth and a transformation to a sigmoidal curve similar to the transformation that occurred with 4-AP alone, as illustrated in Figure 1. All four baroreceptors exposed to 4-AP in combination with phentolamine responded to 4-AP with a decrease in Pth, indicating that α-adrenergic blockade failed to block the effects of 4-AP on baroreceptor excitability. This makes it unlikely that contraction of vascular smooth muscle or enhanced neurotransmitter release from sympathetic nerve terminals in the carotid sinus region was responsible for the changes in baroreceptor discharge observed after 4-AP.

As shown in Figure 4 exposure to TEA did not produce a response like that seen after exposure to 4-AP. Blockade of K+ currents by TEA resulted in a slight increase in slope and Fsat, but no decrease in Pth and no spontaneous activity below Pth as seen after exposure to 4-AP. 4-AP was able to reduce Pth even after prior exposure to TEA (Figure 4), suggesting...
Results

Modeling conductances in these that stimulus-response curves in illustrated characteristics under baroreceptor SIZ curves after result not dal pressure-frequency Figure 6. from zero current injected (Table 2). characteristics remainder of A threshold. For suprathreshold sec was responses subthreshold of five levels of depolarizing injected current of constant amplitude is shown in Figure 6. In each case, the current was stepped up from zero at 5 msec and kept at this new level for the remainder of the run. The model axon generated subthreshold responses at current strengths below threshold. A minimum firing frequency of 12 spikes/sec was found at threshold current of 0.75 μA/cm². For suprathreshold current amplitudes, the axon fired repetitively and generated full-sized action potentials (amplitude = +87 mV) with a spike duration of about 0.4 msec from a resting membrane potential of −70 mV (at 37° C). With increasing current strengths, the firing frequency increased without significant changes in action potential duration or amplitude up to the tested firing frequency of 150 spikes/sec at a current level of 5 μA/cm² (Figure 6). The threshold membrane voltage for action potential onset was about 50 mV. The relation between suprathreshold injected current and firing frequency was fairly linear, as shown in Figure 6, and the threshold frequency of the model axon was comparable with steady-state threshold frequencies found in type I carotid baroreceptors. The model axon was silent at current strengths below threshold and showed a characteristic sudden increase in frequency when the firing threshold current of the SIZ was reached, similar to the sudden onset in firing at P₀ of type I carotid baroreceptors.

To simulate the blocking of K⁺ channels by 4-AP and TEA and the resulting effects on the excitability

Figure 3. Graphs showing the response of a myelinated type I carotid sinus baroreceptor subjected to a ramp increase in carotid sinus pressure during control, exposure to 10⁻⁶ M phentolamine, or a combination of phentolamine and 10⁻⁴ M of 4-aminopyridine (4-AP). Addition of phentolamine to the perfusate did not alter the control curve, nor did it change the characteristic effects of 4-AP on the stimulus-response curve.

that these two drugs block different functional K⁺ conductances in arterial baroreceptors.

4-AP blockade of Iₐ had little or no effect on the stimulus-response curves of type II baroreceptors. As illustrated in Figure 5, this type of baroreceptor is characterized under control conditions by a sigmoidal pressure-frequency relation, similar to type I curves after exposure to 4-AP. Exposure to 4-AP did not result in any significant changes in type II firing characteristics (Table 2).

Modeling Results

The response of the modified H-H model of the baroreceptor SIZ to five levels of depolarizing injected current of constant amplitude is shown in Figure 6. In each case, the current was stepped up from zero at 5 msec and kept at this new level for the remainder of the run. The model axon generated subthreshold responses at current strengths below threshold. A minimum firing frequency of 12 spikes/sec was found at threshold current of 0.75 μA/cm². For suprathreshold current amplitudes, the axon fired repetitively and generated full-sized action potentials (amplitude = +87 mV) with a spike duration of about 0.4 msec from a resting membrane potential of −70 mV (at 37° C). With increasing current strengths, the firing frequency increased without significant changes in action potential duration or amplitude up to the tested firing frequency of 150 spikes/sec at a current level of 5 μA/cm² (Figure 6). The threshold membrane voltage for action potential onset was about 50 mV. The relation between suprathreshold injected current and firing frequency was fairly linear, as shown in Figure 6, and the threshold frequency of the model axon was comparable with steady-state threshold frequencies found in type I carotid baroreceptors. The model axon was silent at current strengths below threshold and showed a characteristic sudden increase in frequency when the firing threshold current of the SIZ was reached, similar to the sudden onset in firing at P₀ of type I carotid baroreceptors.

To simulate the blocking of K⁺ channels by 4-AP and TEA and the resulting effects on the excitability
and firing frequency of the model, we examined three different manipulations of the voltage- and time-dependent characteristics of the K+ currents. The first change involved the time course of the delayed K+ conductance in the subthreshold membrane voltage range. The effects of changing the value of γ₀ on the membrane voltage trajectory are shown in Figure 7A. The axon was made to fire repetitively by injecting depolarizing current with an amplitude of 3 μA/cm². Decreasing the value of γ₀ from the standard value of 2 (heavy black line) to 1 (thin line) resulted in a decrease in action potential amplitude, a reduction in the AHP (from −7 to 0 mV), and a decrease in the interspike interval without a change in action potential duration. The interval preceding the first spike (sometimes called utilization time) was slightly increased by reducing the standard value of γ₀ from 2 to 1. The decrease in interspike interval with a reduction in γ₀ was the result of a reduction in the size of the AHP. The upward shift (toward more depolarized values) of the membrane voltage trajectory during the interspike interval enabled the model axon to reach the voltage threshold for action potential firing sooner than control and thereby increased firing frequency (Figure 7A). The slope of the current-frequency relation of the model was a sensitive function of the value of γ₀, but the threshold current and threshold frequency were not affected by changes in γ₀. Reducing γ₀ from 2 to 1 markedly increased the slope of the curve; increasing the value of γ₀ from 2 to 3 decreased the slope and increased the nonlinearity of the curve (Figure 7B). These results indicate that reduction of the AHP has relatively little effect on the firing threshold but does increase the gain of the spike encoder by increasing the slope of the current-frequency relation.

The second change in model parameters that was investigated was a reduction in δK from the standard value by 50%. This change shortened the interspike interval and, in contrast to a decrease in γ₀, increased action potential duration and peak amplitude (compare panels A and C of Figure 7). This reduction in δK also markedly attenuated the AHP and abolished the negative “dip” immediately following the action.
potential. The reduction in interspike interval with a lower \( g_K \) was mainly the result of the decrease in the size of the AHP. The utilization time was slightly reduced by halving the value of \( g_K \) (Figure 7C). A reduction in the amplitude of \( g_K \) reduced threshold current from 0.75 to 0.50 \( \mu A/cm^2 \) and increased the slope of the current-frequency relation (Figure 7D). A 50% increase in \( g_K \) shifted the curve to the right.

Table 2. Values for Threshold and Saturation Pressures, Threshold and Saturation Firing Frequencies, and Slopes for Type II Baroreceptors in Response to Exposure to 4-Aminopyridine

<table>
<thead>
<tr>
<th></th>
<th>Control (n=5)</th>
<th>( 10^{-5} ) M (n=5)</th>
<th>( 5 \times 10^{-5} ) M (n=5)</th>
<th>( 10^{-4} ) M (n=5)</th>
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<tr>
<td>Threshold pressure (mm Hg)</td>
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<td>Saturation pressure (mm Hg)</td>
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<td>183.4±15.6</td>
<td>182.8±15.0</td>
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<tr>
<td>Threshold firing frequency (spikes/sec)</td>
<td>5.8±2.8</td>
<td>4.6±1.9</td>
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<tr>
<td>Saturation firing frequency (spikes/sec)</td>
<td>21.2±6.4</td>
<td>22.2±5.7</td>
<td>21.6±5.3</td>
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<tr>
<td>Slope (spikes/sec/mm Hg)</td>
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<td>0.27±0.12</td>
<td>0.25±0.11</td>
<td>0.24±0.10</td>
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Values are mean±SEM. No significant differences were found for any level of 4-aminopyridine exposure.
with minor effects on the slope. These results indicate that blockade of $I_K$ by reducing its conductance has only small effects on the firing threshold but can increase the slope of the current-frequency curve.

Decreasing the value of $g_A$ by 5% from the standard value resulted in a shortened interspike interval, but no appreciable change in action potential duration, peak amplitude, or size of the AHP was observed (Figure 7E). The utilization time was markedly decreased by this manipulation of $g_A$. In contrast to the changes in the voltage- and time-dependent characteristics of $I_K$ described above, the shortened interspike interval in response to a decrease in $g_A$ was largely the result of an increase in the slope of the interspike membrane depolarization. The firing threshold of the current-frequency relation was sensitive to small changes in $g_A$ as shown in Figure 7F. A 5% reduction in $g_A$ shifted the curve to the left and slightly reduced the slope. The model axon became spontaneously active with no injected current. The curve was shifted to the right by a 5% increase in $g_A$, increasing the threshold current from 0.75 to 2.0 $\mu$A/cm$^2$, without affecting the slope or the threshold firing frequency (Figure 7F). Compared with reductions in the size of the AHP (through a decrease in $\gamma_h$) or $g_{K_0}$, a decrease in $g_A$ had a much more pronounced effect on the threshold current of the model axon. This indicates that $g_A$ normally functions to reduce the responsiveness of the axon to small stimuli and that reduction of $g_A$ by only a small amount results in spontaneous activity. In carotid baroreceptors, the most conspicuous effect of exposure to 4-AP was a decrease of the firing threshold without an increase in the slope of the stimulus-response curve, indicating that these effects were probably mediated through suppression of $I_A$, with no or weak effects on $I_K$. In
contrast, TEA had little effect on the firing threshold but increased the slope of the baroreceptor stimulus-response relation, consistent with suppression of $I_R$.

To further illustrate the relation between the experimental and modeling results regarding the effects of 4-AP on carotid baroreceptor discharge, a schematic representation of the mechanoelectrical transduction process in carotid myelinated baroreceptors is shown in Figure 8. In this figure, this process is described as a multistep mechanism, similar to the one proposed for other types of mechanoreceptors.27 We assumed that the spike-encoding step in carotid baroreceptors was characterized by a linear relation between depolarizing membrane current and firing frequency and, therefore, that most of the nonlinearities in the static baroreceptor pressure-frequency curve above $P_{th}$ could be attributed to nonlinearities in other steps of the mechanoelectrical transduction process preceding the encoding step. The basic steps include 1) the input signal, consisting of a slow ramp increase in CSP, 2) the increase in sinus wall strain resulting from pressure deformation as a nonlinear saturating function of CSP,28 3) an increase in sinus wall strain leading to a proportional increase in receptor deformation for suprathreshold sinus wall strain (the threshold is determined by the elastic modulus of the sinus wall elements—smooth muscle, collagen, and elastin—to which the receptor is coupled and by the coupling mechanism itself),1 4) receptor deformation leading to a proportional increase in the generator or transducer current by analogy to the linear relation found between receptor deformation and transducer current in other types of mechanoreceptors,3–5 and 5) the transducer current spreading electrotonically to the SIZ, with the firing frequency a linear function of the transducer current (for transducer current $\geq$ threshold current) as derived from our modeling studies. The relation between CSP and baroreceptor firing frequency (step 6 of Figure 8) reflects the characteristics of the individual steps and shows schematically the recorded type I stimulus-response relation (thin line). The heavy line in steps 5 and 6 of Figure 8 shows the effects of reducing $g_A$ on the current-frequency curve as described by our model. This shift to the left in step 5 causes a shift to the left in the pressure-frequency relation (step 6), similar to the change in stimulus-response curves after 4-AP exposure. The $P_{th}$ under these conditions reflects the mechanical threshold of step 3. The spontaneous activity of the baroreceptor below $P_{th}$ is determined by

**Figure 7.** Graphs showing voltage trajectories of the model spike-initiation zone in response to injected current (3 $\mu$A/cm²) (panels A, C, and E) and the corresponding steady-state current-frequency relations (panels B, D, and F). Control action potentials on the left of the figure are indicated by heavier black lines. Also shown are the effects on action potential shape and impulse firing of reducing the time constant multiplication function at the resting membrane potential ($\gamma_d$) from the standard value of 2 to 1 (panel A; thin lines), reducing maximum delayed outward $K^+$ conductance ($gK$) by 50% from its standard value (panel C; thin lines), and reducing maximum transient $K^+$ conductance ($gA$) by 5% from its standard value (panel E; thin lines). Shown on the right of the figure are the effects on the static current-frequency relation of increasing $\gamma_d$ of maximum $gK$ to 3 or decreasing $\gamma_d$ of maximum $gK$ to 1 from the standard value ($\gamma_d=2$) (panel B), the effects of increasing or decreasing maximum $gK$ by 50% from its standard value (panel D), and the effects of increasing or decreasing maximum $gA$ from its standard value by 5% (panel F). Curves in panels B, D, and F are drawn as best-fit solutions to points generated for control ($^*$), increases (+), and decreases (-) in the respective parameters.
the firing threshold of the spontaneously firing SIZ in the absence of a mechanical stimulus.

Discussion

In our accompanying article in this journal, it was shown that carotid baroreceptors can be divided into two groups based on their discharge pattern in response to a ramp increase in CSP. Type I baroreceptors abruptly start firing when their \( P_{th} \) is reached, followed by an initially rather steep increase in firing rate for suprathereshold pressures to a plateau at saturation pressure. Type II baroreceptors are spontaneously active until \( P_{th} \) is reached, above which they show a gradual increase in firing with further increases in pressure up to saturation. In this paper, we investigated whether the electrophysiological properties of the baroreceptor membrane at the SIZ contribute to these differences in stimulus-response curves. Because the receptor endings themselves are too small to permit intracellular recording of ionic currents, we used a combination of pharmacological blockade and computer modeling to examine the role played by \( K^+ \) currents in regulation of baroreceptor excitation. Our results indicate that \( I_A \) is responsible for keeping type I neurons quiescent at low CSP, especially in those receptors with a high \( P_{th} \). In contrast, \( I_A \) seems to play only a minor role in regulation of the firing of type II receptors. Our experiments suggest that the wide range of \( P_{th} \)s found among carotid baroreceptors depends in part on differences in relative importance of \( I_A \).

Our results are consistent with other studies showing that pharmacological blockade of \( K^+ \) channels by 4-AP leads to an increase in excitability and spontaneous pacemaker discharge in previously silent neurons.11,13,14,29 In a variety of preparations, 4-AP acts as a relatively selective blocker of \( I_A \) when applied in concentrations in the low millimolar range or less.11-13,15,30,31 Higher concentrations of 4-AP can also block the \( I_C \).12,13,15 \( I_K \) in vertebrate neurons is generally more sensitive to blockade by TEA than by 4-AP.13,15 However, pharmacological separation of \( I_A \) and \( I_K \) by 4-AP and TEA is not always possible, as shown in rat sympathetic ganglia.32 Thus, sensitivity to the blocking actions of 4-AP varies considerably between cell types: half-maximal blockade occurs at micromolar concentrations in some neurons, but in other cells blockade does not occur until millimolar concentrations of 4-AP are applied.15 It has been suggested that these differences in sensitivity to 4-AP are related to the existence of different subclasses of \( I_A \) channels, each with different voltage- and time-dependent kinetics.15 \( I_A \) with slow inactivation kinetics are generally more sensitive to 4-AP,14,15,30 and it could be that \( I_A \) in carotid baroreceptors has the same type of slow kinetics based on similarities in drug sensitivity. It has been shown that 4-AP has little effect on other modulatory \( K^+ \) currents, such as the \( Ca^{2+} \)-activated \( K^+ \) current and the M current, or \( Ca^{2+} \) currents.31 Single-channel recordings showed that 4-AP acts on \( I_A \) channels from the inside to reduce both channel conductance and open time.23 Our results regarding baroreceptor sensitivity to 4-AP, the reversibility of its actions, and effects on receptor discharge are consistent with responses that can be attributed to a selective blockade of \( I_A \) in carotid baroreceptors. The absence of significant effects of TEA on firing threshold supports this hypothesis.

Despite the variations in kinetics and differences in sensitivity to 4-AP, most A currents seem to have similar functional roles (namely, to regulate repetitive firing and to dampen excitability).6,10,12,15 Comparisons of firing patterns and membrane properties of dif-
ferent crab axons have shown that only those cells with a pronounced I_A were capable of firing at a low repetitive rate. Neurons that lacked this current were incapable of firing repetitively. Similar results have been obtained in mammalian sympathetic ganglion cells. However, in some neurons, I_A has also been shown to be involved in spike repolarization. Thus, the relative contribution of the transient K^+ conductance to the total membrane conductance has been shown in a number of preparations to be of considerable importance in shaping the different responses of neurons to excitatory stimuli, similar to the results obtained in this study.

The effects of 4-AP on carotid baroreceptor discharge are unlikely to be the result of possible indirect effects of this drug on carotid sinus distensibility or sympathetic neurotransmitter release. It is conceivable that 4-AP could alter baroreceptor firing through blocking actions on I_A currents that have been described in smooth muscle or in cultured vascular endothelial cells. In endothelial cells, one third of the cells develop A-like currents in secondary culture that can be blocked by 5 mM of 4-AP. It is not known if lower concentrations of 4-AP are effective in blocking I_A in these cells or what the functional importance of these findings is in vivo. Guinea pig pulmonary artery smooth muscle cells are more sensitive to 4-AP than most other types of smooth muscle cells and contract when exposed to 10^-5 M of 4-AP. It was suggested that 4-AP exerts its effects on smooth muscle tone through enhanced sympathetic neurotransmitter release, because this effect can be blocked by prior α-adrenergic blockade with phentolamine. Phentolamine also blocks the sensitizing effects of sympathetic neurotransmitters on baroreceptor discharge. In our study, the actions of 4-AP alone and in combination with phentolamine were similar, indicating that the effects of 4-AP were not mediated through these indirect mechanisms. Furthermore, we did not observe a change in CSP-volume relations during ramp pressure changes after addition of 4-AP to the sinus perfusate (Figure 9), indicating that the distensibility of the carotid sinus region was not altered by this drug. However, it is possible that small changes in the microenvironment of the receptor endings occurred that we were not able to detect.

Although we did not test directly for the site of action of 4-AP in this study, we believe that it was most likely at or near the site where spike initiation is thought to occur in arterial baroreceptors (namely, in the naked, unmyelinated endings themselves). Myelinated baroreceptors gain a myelin sheath central to this site in the outer adventitia of the sinus wall. It is known that the nodal membrane of mammalian myelinated axons does not have K^+ channels and that 4-AP is without effect in mature myelinated axons. In addition, it has been shown in both mammalian and frog myelinated axons that the nodal membrane is unable to fire repetitively in response to a constant depolarizing stimulus. This suggests that the spike-encoder region on the baroreceptor axon has unique properties that differ from the nodal membrane further along the axon. In the Mauthner cell of the goldfish it has been possible to obtain independent recordings from the SIZ on the axon hillock and the axon distal to this site. These cells it has been confirmed experimentally that the SIZ possesses K^+ channels that can be blocked by intracellularly applied TEA and 4-AP and that these channels are lacking further along the axon in the nodal membranes.

Although I_A has been described as a characteristic feature of repetitively firing neurons, information about I_A in slowly adapting mechanoreceptors is scarce. In the stretch receptor of the lobster, both 10^-2 M TEA and 5 x 10^-4 M of 4-AP reduced the rate of spike repolarization and caused depression of the AHP by blocking a slowly inactivating outward current. Neither drug alone was able to block the receptor K^+ current, suggesting that more than one K^+ current, including a current similar to I_A, could be present in these neurons.

In the absence of voltage-clamp data, modeling of the membrane properties of the SIZ represents an alternative approach to the understanding of repetitive firing of arterial baroreceptors. The H-H model has proved to be a widely accepted model for the description of action potential generation in both invertebrate and vertebrate neurons and in a modified form has successfully been applied to simulate the impulse discharge in invertebrate slowly adapting mechanoreceptors. This model has been particularly well studied and has provided us with a useful starting point for the development of a model for...
spike encoding in baroreceptors. However, the steady-state current-frequency relation of the H-H model is characterized by a rather high minimum frequency, a limited frequency modulation range, and a nonlinear current-frequency curve for supra-threshold current. For instance, the minimum frequency of the H-H model of the squid axon at a temperature of 25°C lies well above 200 Hz. In contrast, many transducer-encoder neurons, including arterial baroreceptors, can fire repetitively at much lower frequencies and show a substantial frequency modulation range. The addition of $I_A$ to the H-H axon and the increase in the spike AHP through modification of the time constant of $I_K$ enabled the H-H axon to function as a spike encoder, consistent with the results obtained by others. As in some other repetitively firing neurons, $I_K$ in our model was substantially nonactivated at subthreshold and near-threshold membrane potentials. $I_A$ is inactivated by the membrane depolarization during the spike portion of the action potential and is restored by the AHP following the spike. The resultant transient outward current slows down the return of the membrane potential toward action potential threshold, thereby prolonging the interspike interval. In addition, a small or brief depolarization will be immediately opposed by $I_A$, which dampens excitability and keeps neurons from firing spontaneously. In agreement with previous studies, we found that the threshold current of the spike-encoding process was dependent on the membrane parameters for the $I_A$ and that the properties of $I_K$ were important in determining the slope of the current-frequency relation (Figure 7). Suppression of $I_A$ by reducing the value of $g_K$ was much more effective in lowering the threshold current than a 10-fold larger reduction in $g_K$ (Figure 7, panels D and F). The lowering of the threshold current by reducing $g_K$ paralleled a marked reduction in the utilization time (Figure 7E), illustrating that the strong inactivation kinetics of $I_A$ normally function to delay the onset of action potential generation. The computer simulations also show the importance of the interaction between $I_A$ and $I_K$ during the spike AHP as first noted by Connor et al. As shown in Figure 7C, a reduction in $g_K$ removes the spike undershoot immediately after the action potential, and this results in less reactivation of the $I_A$ system. The small decrease in threshold current seen after a reduction in $g_K$ (Figure 7D) is therefore most likely the result of an associated decrease in $I_A$. The modeling results provide additional support for the hypothesis that only a small reduction in $g_K$ is needed to change the firing threshold of the SIZ under conditions in which $I_A$ is only partially inactivated at the resting membrane potential. This might explain the relatively large sensitivity of myelinated carotid baroreceptors to the blocking actions of 4-AP.

The $P_{th}$ of arterial baroreceptors is determined by a combination of factors (see Figure 8), including sinus wall mechanics, the coupling between receptor endings and sinus wall elements, and baroreceptor electrophysiological properties. Our results indicate that the presence or absence of spontaneous discharge at low CSPs and the sharp "jump" in frequency at $P_{th}$ depend in part on the differences in voltage- and time-dependent kinetics of $I_A$. Initial results regarding the lack of effect of 4-AP on type II receptors indicate that the magnitude or kinetics of $I_A$ in type II receptors are such that, in these cells, $I_A$ is unable to prevent spontaneous activity below the $P_{th}$. It is interesting to note that, in type I baroreceptor fibers that became spontaneously active after exposure to 4-AP, an apparent dissociation between firing threshold of the SIZ and the $P_{th}$ was observed (see Figure 1). Under control conditions, this baroreceptor started to fire action potentials in response to ramp CSP at about 85 mm Hg. With $10^{-4}$ M of 4-AP, the $P_{th}$ decreased to 50 mm Hg, but firing threshold of the SIZ, indicated by appearance of spontaneous activity, was lowered to 10 mm Hg. Under these conditions, when mechanical and electrophysiological thresholds were uncoupled, it became apparent that this baroreceptor was able to respond to sinus wall stretch starting at a pressure of 50 mm Hg, but the SIZ was capable of spontaneous firing below that pressure due to blockade of $I_A$ by 4-AP. However, under control conditions, this type I receptor was kept silent until a much higher pressure was reached, presumably by the damping actions of $I_A$ on SIZ excitability. This mechanism could therefore be responsible for the higher $P_{th}$ in type I compared with type II receptors as described in our accompanying paper.

Undoubtedly our model represents an oversimplification of the actual spike-encoding process as it occurs in arterial baroreceptors. Other membrane parameters such as the amplitude and kinetics of sodium conductance, leakage conductance, and membrane capacitance are also important in the determination of threshold, gain, and modulation range of the encoding step. In recent years, studies in invertebrate mechanoreceptors have demonstrated the presence of voltage-dependent $Ca^{2+}$ currents, $Ca^{2+}$-activated $K^+$ currents, an electrogenic Na-K pump, and slow Na+ and $K^+$ inactivation, which might be important in frequency adaptation. Indirect evidence indicates that some of these membrane characteristics are also present in arterial baroreceptors. Intracellular recordings from the cell bodies of carotid baroreceptors located in the petrosal ganglion have shown that at least three types of baroreceptor cells exist, based on differences in passive electrical membrane properties and ionic currents that could reflect differences in electrophysiological characteristics of their peripheral endings. Therefore, further studies are needed to determine more precisely the electrophysiological properties of type I and type II baroreceptor endings.
Appendix
The following symbols and nomenclature were used in the modeling studies:

- \( V_m \): Membrane potential (inside vs. outside)
- \( I_m \): Membrane current per unit area
- \( C_m \): Membrane capacitance per unit area
- \( I_C \): Capacitative current per unit area
- \( I_i \): Ionic current per unit area
- \( I(t) \): Stimulus current per unit area
- \( I_{th} \): Minimum current necessary to elicit action potentials under steady-state conditions
- \( g_{Na}, g_K, g_L, g_A \): Membrane conductances for sodium, potassium, leakage, and transient potassium channels
- \( g_{Na}\prime, g_K\prime, g_A\prime \): Maximum values for \( g_{Na}, g_K, g_A \)
- \( m, n, h, A, B \): Activation and inactivation parameters for ionic channels
- \( m_s, n_s, h_s, A_s, B_s \): Steady-state values for \( m, n, h, A, B \)
- \( \alpha_m, \alpha_n, \alpha_h, \beta_m, \beta_n, \beta_h \): Rate constants for activation and inactivation parameters
- \( \tau_m, \tau_n, \tau_h, \tau_A, \tau_B \): Time constants for activation and inactivation parameters
- \( I_{Na}, I_K, I_L, I_A \): Sodium, delayed potassium, leakage, and transient potassium currents
- \( E_{Na}, E_K, E_L \): Equilibrium potentials for sodium, potassium, and leakage currents
- \( [Na]\), [Na]\_0: Intracellular and extracellular sodium concentration
- \( [K]\), [K]\_0: Intracellular and extracellular potassium concentration
- \( \gamma \): Time constant multiplication function
- \( \gamma_0 \): Value of \( 1/\gamma \) at \( V_m = -60 \text{ mV} \)

According to Hodgkin and Huxley,\(^8\) the instantaneous current-voltage relation is linear in voltage-clamped squid axons, and under normal ionic conditions the total current that flows across the axon membrane during an action potential (\( I_m \)) equals the sum of the capacitative current (\( I_C \)) and the ionic current (\( I_i \)):

\[
I_m = I_C + I_i = C_m (dV_m/dt) + I_i
\]

where \( C_m \) is the membrane capacitance and \( dV_m/dt \) the change in membrane potential. The current in open sodium and potassium channels (\( I_{Na} \) and \( I_K \)) can be described by

\[
I_{Na} = g_{Na}(V_m - E_{Na})
\]

\[
I_K = g_K(V_m - E_K)
\]

where \( E_{Na} \) and \( E_K \) are the \( Na^+ \) and \( K^+ \) equilibrium potentials and \( g_{Na} \) and \( g_K \) are the membrane conductances for \( Na^+ \) and \( K^+ \), respectively. Included in the H-H model of the axon membrane is also a leakage conductance (\( g_L \)) in series with a leakage battery (\( E_L \)). \( g_L \) is independent of \( V_m \). \( I_m \) underlying the action potential can be described as

\[
I_m = I_C + I_{Na} + I_K + I_L
\]

\[
I_m = C_m(dV_m/dt) + g_{Na}(V_m - E_{Na}) + g_K(V_m - E_K) + g_L(V_m - E_L)
\] (A1)

The conductances \( g_{Na} \) and \( g_K \) can be represented as the product of a maximum conductance (\( g_{Na}\prime \) and \( g_K\prime \), respectively) and a multiplying factor representing the fraction of the maximum conductance actually expressed. In the H-H model, these coefficients are described by the parameters \( m, n, h, A, B \), which vary between 0 and 1. The transient kinetics of \( g_{Na} \) and \( g_K \) can be fitted by

\[
I_{Na} = m^3 h g_{Na}(V_m - E_{Na})
\]

\[
I_K = n^4 g_K(V_m - E_K)
\]

The voltage and time dependency of the H-H model parameters are satisfied by the differential equation:

\[
dx/dt = \alpha (1 - m) - \beta m
\]

where \( m, n, h, A, B \) are the voltage-dependent rate constants for \( m, n, h \), and \( A, B \). Equation A1 can be rewritten as

\[
I_m = m^3 h g_{Na}(V_m - E_{Na}) + n^4 g_K(V_m - E_K)
\]

\[
+ g_L(V_m - E_L) + C_m(dV_m/dt)
\]

The transient potassium current (\( I_A \)) can be expressed in terms of the functions used in the H-H model as\(^8\)

\[
I_A = g_A(V_m - E_K)
\]

with \( g_A = g_{A'} A^3 B \). The \( A \) and \( B \) factors have the same functional significance as the \( m \) and \( h \) parameters of the sodium conductance system.\(^8\) The governing differential equation for the total \( I_m \) of the space-clamped axon is

\[
I(t) = \begin{aligned}
I_m &= g_{Na} m^3 h (V_m - E_{Na}) + g_K n^4 (V_m - E_K) + \\
&+ g_L (V_m - E_L) + g_A A^3 B (V_m - E_K) + C_m (dV_m/dt)
\end{aligned}
\] (A2)

where \( I(t) \) is the stimulating current. Solving Equation A2 for \( dV_m/dt \),

\[
dV_m/dt = (1/C_m)(I(t) - g_{Na} m^3 h (V_m - E_{Na}) - \\
g_K n^4 (V_m - E_K) - g_L (V_m - E_L) - g_A A^3 B (V_m - E_K))
\]
Squid axon data was used as the basis for constructing the m and h functions, and crustacean leg axon data was used to calculate the n, A, and B functions, similar to the description for the crustacean leg axon proposed by Connor. A Q10 of 3 was used to express the temperature dependence of the rate constants of the ionic conductances, and a Q10 of 1 was used for the conductances themselves. Q10 represents the ratio of a measured value at one temperature to the value at a temperature 10° lower. The rate constants are related to the time constants and the steady state values of activation and inactivation as follows:

\[ \tau_a = \frac{1}{(\alpha_a + \beta_a)} \]

\[ x = \frac{\alpha_a}{(\alpha_a + \beta_a)} \]

where \( x \) stands for m, n, or h and \( x \) represents the steady-state value of \( x \).

The following expressions and parameter values were used to construct the amplitude and time course of the Iₜs:

**Equations**

1) Sodium conductance (g₅₄ₙₐ)

- a) For m,
  \[ \alpha_m = 0.1(V_{m} + 35)/(1 - \exp[-(V_{m} + 35)/10]) \]
  \[ \beta_m = 4\exp[-(V_{m} + 60)/18] \]

- b) For h,
  \[ \alpha_h = 0.07\exp[-(V_{m} + 60 + \text{hshft})/20] \]
  \[ \beta_h = 1/[1 + \exp[-(V_{m} + 30 + \text{hshft})/10]] \]

where hshft represents the value by which the steady-state gating variable \( h \) was shifted toward more depolarized values along the voltage axis (hshft = −6 mV).

2) Delayed potassium conductance (gₜₙ)

- a) \( \tau_n \) was multiplied by a voltage-dependent function (\( \gamma \)) such that
  \[ \tau_n^* = (1/\gamma)\tau_n \]

\( g_k \) varies linearly with \( V_{m} \) such that

\[ \gamma = 1/\gamma_0 - \{(1 - 1/\gamma_0)(V_{m} - 60)/(E_{Na} + 60)\} \]

where \( \gamma_0 \) is the value of \( \gamma \) at the resting \( V_m \). When \( V_m = -60 \text{ mV} \), \( \gamma = 1/\gamma_0 \); when \( V_m = E_{Na} \), then \( \gamma = 1 \).

- b) For n,
  \[ \alpha_n = 0.01(V_{m} + 50 + \text{nshft})/(1 - \exp[-(V_{m} + 50)/10]) \]
  \[ \beta_n = 0.125\exp[-(V_{m} + 60 + \text{nshft})/80] \]

with \( \alpha_n^* = \gamma_0 \alpha_n \) and \( \beta_n^* = \gamma \beta_n \). The \( n \) curve was shifted 4 mV to the right on the voltage axis (nshft = −4).

3) Transient potassium conductance (gₐₙₐₗₜ)

\[ A_n = [0.0761 \exp[(V_{m} + 94.22)/(31.84)] + \exp[(V_{m} + 1.17)/(28.93)])^{1/3} \]

\[ \tau_A = 0.3632 + 1.158/[1 + \exp((V_{m} + 55.96)/20.12)] \]

\[ B_n = 1/[1 + \exp[(V_{m} + 53.3)/(14.54)]^{1/3} \]

\[ \tau_n = 1.24 + 2.678/[1 + \exp[(V_{m} + 50)/(16.027)] \]

where \( \tau_n (dA/dt) + A = A_n \), and \( \tau_0 (dB/dt) + B = B_n \).

**Constants**

- a) H-H values were used for \( C_m \) and \( g_{ti} \): \( C_m = 1 \mu F/cm^2 \); \( g_{ti} = 0.3 \text{ mmho/cm}^2 \).
- b) It was necessary to alter the value of the leakage battery \( E_k \) from the usual value to +13 mV (37° C) to counterbalance the nonactivated \( I_A \) at the resting membrane potential and satisfy the initial conditions \( dV_m/dt = 0 \) and \( I_m = 0 \). This is analogous to making the leakage channel more selective for sodium.
- c) \( g_{K} \) was set at its usual value of 120 mmho/cm² (H-H), and \( E_{Na} \) was calculated from the Nernst equation with \([Na]_l = 1.154 \text{ mM} \) and \([Na]_s = 15 \text{ mM} \).
- d) \( g_{K} \) was reduced from 36 (H-H) to 20 mmho/cm².
- e) \( E_k \) was calculated from the Nernst equation with \([K]_s = 4 \text{ mM} \) and \([K]_l = 140 \text{ mM} \).
- f) The standard value of \( g_{K} \) was reduced from 47.7 to 20 mmho/cm² to limit the increase in \( I_h \) resulting from a large \( I_A \).
- g) The reversal potential for \( g_{K} \) was set equal to \( E_k \).

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