Mathematical description of regenerative potentials recorded from circular smooth muscle of guinea pig antrum

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Edwards, F. R., and G. D. S. Hirst. Mathematical description of regenerative potentials recorded from circular smooth muscle of guinea pig antrum. Am J Physiol Gastrointest Liver Physiol 285: G661–G670, 2003. First published June 4, 2003; 10.1152/ajpgi.00145.2003.—Regenerative potentials evoked by intracellular current injection in single bundles of circular smooth muscle taken from guinea pig antrum have the characteristics of the secondary regenerative component of the slow wave occurring in the same muscle layer. Such regenerative depolarizations might result from a mechanism that responds to membrane polarization with a delayed increase in the rate of production of unitary potentials detected in this tissue. To test this possibility, a two-stage reaction leading to the formation of an intracellular messenger was proposed. The first forward reaction was voltage-dependent, in the manner described by the Hodgkin-Huxley transient Na conductance formalism, allowing simulation of anode break excitation, stimulus threshold strength-duration characteristics, and refractory behavior. A conventional dose-effect relationship was proposed to describe the dependence of the mean rate of discharge of unitary potentials on messenger concentration. Unitary potentials were modeled as unitary membrane conductance modulations with an empirically derived amplitude distribution and Poisson-distributed intervals. The model reproduces a range of spontaneous and evoked membrane potential changes characteristic of antral circular muscle bundles.

Regenerative responses were consistent with a cascade of unitary potentials rather than the activation of sets of voltage-dependent ion channels (6). Depolarizing current evokes regenerative responses with spectral profiles similar to those of the membrane noise but having higher levels of power. The spectral properties of regenerative responses were consistent with a cascade of unitary potentials rather than the activation of sets of voltage-dependent ion channels (6).

This model tests the possibility that regenerative responses are summations of many unitary membrane conductance modulations that arise at random times and that the mean time between unitary potentials becomes brief after activation of a voltage-sensitive messenger pathway.

MODEL FORMULATION

Formation of messenger depends on $E_m$. As pointed out, regenerative potentials appear to result from voltage-dependent formation of a messenger, followed by release of Ca$^{2+}$ from internal stores and subsequent activation of Ca$^{2+}$-sensitive anion channels. The fundamental postulate defining the model is the reaction chain that leads from a change in $E_m$ to formation of a messenger substance, presumably Ins(1,4,5)P$_3$. A single reaction step with a voltage-sensitive forward reaction rate was considered first. Hodgkin and
Huxley (15) were able to employ a convenient mathematical device to model the brief delay preceding the Na conductance change in the squid axon. They proposed that the conductance was proportional to the third power of its activation variable, which obeyed a first-order differential equation. In the case of antral regenerative potentials, latency is not brief but long, compared with rise time. A power very much larger than three would be required to simulate the latency.

The long latencies seen in this tissue were alternatively modeled as a two-stage chemical reaction. Voltage sensitivity was applied to the forward rate constant \( (m h K_F) \) for the first reaction step, presumably occurring at the membrane, which converts a precursor molecule to an intermediate reagent. The activation variable was designated \( m \), and the inactivation variable, \( h \); these designations were borrowed from Hodgkin and Huxley’s (15) squid axon Na current description. The first reaction step was represented as a reversible reaction with the reverse rate constant \( (K_B) \). The second step from the intermediate reagent to the messenger proceeded with net formation rate \( (K_M) \), and the messenger was proposed to hydrolyze with the rate constant \( (K_H) \) as shown.

\[
\text{[precursor]} \xrightarrow{m h K_F} \text{[intermediate]} \xrightarrow{K_M} \text{[messenger]}
\]

The equations describing the variations in intermediate concentration ([intermediate]) and messenger concentration ([messenger]) were

\[
\frac{dm}{dt} = mhK_F[\text{precursor}] - KM[\text{intermediate}]
\]

\[
\frac{dh}{dt} = K_M[\text{intermediate}] - hK_B[m][\text{messenger}]
\]

where the \( K_F \) [precursor] = 0.0157, \( K_B = 3.3 \), \( K_M = 0.4478 \), and \( K_H = 0.3 \).

The following equations were used to calculate \( m \) and \( h \).

\[
\frac{dm}{dt} = m_m - m
\]

\[
\tau_m = m = 1/(1 + e^{S_m(V_m-V_m^c)})
\]

\[
\frac{dh}{dt} = h_s - h
\]

\[
\tau_h = h = 1/(1 + e^{S_h(V_h-V_h^c)})
\]

where \( S_m = 0.3 \); \( V_m = -59.5 \); \( S_h = 0.32 \); \( V_h = -67.5 \); \( \tau_m = 1.2 \); \( \tau_h = 6 \); \( S_i = 0.5 \); and \( V_i = -45.5 \).

These equations are illustrated in Fig. 1A, which shows steady-state activation and inactivation values \( (m_a \) and \( h_a \) and associated time constants \( (\tau_m \) and \( \tau_h \) \) as functions of \( E_m \).

Initial conditions were: \( E_m = -65 \text{ mV} \); [messenger] = 340 nM; [intermediate] = 220 nM; [m] = 0.18; and \( h = 0.28 \).

Discharge rate of unitary potentials depends on [messenger]. The mean rate of unit discharge \( (\lambda) \) was proposed to depend on the concentration of the intracellular messenger according to the following dose-effect relationship, which is shown graphically in Fig. 1B.

\[
\lambda = \frac{\lambda_{max}[\text{messenger}]}{K_D + [\text{messenger}]^H}
\]

The maximum mean rate of unit discharge \( (\lambda_{max}) \) was 140 Hz in control conditions, the Hill coefficient \( (H) \) was 6.8, and the affinity \( (K_D) \) was 750 nM. Although the value chosen for \( K_D \) was arbitrary because changes in \( \lambda \) could be compensated by adjustment of \( K_M \) (Eq. 1), 750 nM is a value reported for a mammalian Ins(1,4,5)P_3 receptor (23) and is within the range (100 to 1,000 nM) offered by Marchant and Taylor (22) for Ins(1,4,5)P_3 receptors in hepatocytes.

Regenerative potentials are composed of discrete unitary potentials. The spontaneous unitary membrane depolarizations seen in recordings of intracellular potential made in this tissue have been previously characterized (6). Individual unitary depolarizations were well fitted by the difference between two exponential functions raised to the third power. The intervals between successive unitary potentials were approximately Poisson distributed. Therefore, the spectral density in the region between –0.1 and 20 Hz can be described by a curve whose gradient approaches a theoretical extreme of –8 at high frequencies. See Edwards et al. (6) for full description.

Unitary potentials were assumed to reflect unitary membrane conductance increases having a similar time course. The equilibrium potential for the current they carry, obtained by extrapolation, is close to –20 mV (13). Clearly, although this may not be a measure of the true reversal potential, for example, if rectification occurs at more positive potentials, the extrapolated value of reversal potential is appropriate for potential changes occurring in the physiological ranges from which the extrapolated value was obtained. If a particular unitary conductance modulation, \( g(t) \), begins at time \( t_i \) and has amplitude \( A_0 \), it can be represented as follows (6).
\[ g_i(t) = 0, \quad t < t_j \]
\[ g_i(t) = A_i e^{-\frac{t-t_j}{0.434}} - e^{-\frac{t-t_j}{0.077}}, \quad t \geq t_j \]  

(4)

The intervals between the starting times of unitary conductance modulations were the average values obtained from 17 experiments (6). This is an exponentially smoothed version of the frequency function shown in Fig. 9D of Edwards et al. (6).

The total \( g_u(t) \) is the sum of the \( N \) individual unitary modulations that begin before \( t \)

\[ g_u(t) = \sum_{j=1}^{N} g_j(t) \]  

(5)

The time constants 0.434 and 0.077 s used in these calculations are the average values obtained from 17 experiments (6). \( A_i \) is an instance of a random variable whose frequency distribution is shown in Fig. 2A. This is an exponentially smoothed version of the frequency function shown in Fig. 9D of Edwards et al. (6).

The intervals between the starting times of unitary conductance modulations were drawn from a Poisson distribution (6). The mean frequency of unit discharge is by definition the inverse of the mean interval between units, and is given by

\[ \lambda = \frac{N}{\sum_{j=1}^{N} (t_j - t_{j-1})} \]  

(6)

At each integration step, \( \lambda \) was recalculated from the concurrent value of \( \lfloor \text{message} \rfloor \) (Eq. 3) and the intervals between forthcoming unitary conductance modulations were rescaled accordingly.

Electrical equivalent cell. A short segment of a single bundle of circular muscle from guinea pig antrum comprises \( \sim 200 \) ICCm\(_m\) distributed throughout a syncytium of \( \sim 2,000 \) smooth muscle cells. The ICCm\(_m\) are syncytially interconnected and make electrical connections with many surrounding muscle cells (26). When a segment of a single circular muscle bundle was impaled with two microelectrodes, the recordings were always very similar even when the electrodes were hundreds of micrometers apart. Within a bundle, voltage responses to injected current pulses never differed by >5% in amplitude, regardless of electrode position and separation. Moreover the entire time course of an electrotonic potential could be described by a single exponential function. These observations suggest that the intracellular compartment within a muscle bundle can be considered electrically isopotential and that a bundle can therefore be represented electrically as a single equivalent cell. The equivalent cell membrane was represented as the parallel combination of the total unitary conductance \( g_u(t) \) at \( t \) with an equilibrium potential equal to the extrapolated reversal potential for unitary events (\( E_u \)), an aggregate linear background conductance \( g_b(t) \) with an equilibrium potential equal to the resting potential \( (E_b) \) and a capacitor representing membrane capacitance \( (C_m) \) (Fig. 2B).

Therefore, the resistive membrane current \( (i_m) \) was represented as the sum of two components. The first was the current carried by the total unitary conductance modulation with an equilibrium potential of \( \sim 20 \) mV. The second was an equivalent net background current with an equilibrium potential of \( \sim 65 \) mV and a conductance of 227 nS to agree with the mean input resistance of preparations of 4.4 M\( \Omega \) [Table 1, Edwards et al. (6)].

\[ i_m = g_u(t)(E_u + 20) + 227(E_b + 65) \]  

(7)

The variation in \( E_m \) is given by

\[ \frac{dE_m}{dt} = -\frac{i_m}{C_m} \]  

(8)

where \( C_m \) is the total membrane capacitance of the circular muscle bundle preparation. The mean membrane time constant of circular muscle preparations was 160 ms (28), and

\begin{table}[h]
\centering
\caption{Tolerances for individual model parameters}
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Parameter & Equation & Units & Base Value & Increase, % & Reduction, % \\
\hline
\( K_f \) [precursor] & 1 & mM/s & 0.0157 & 10 & 5 \%
\hline
\( K_b \) & 1 & s\(^{-1}\) & 3.3 & 5 & 4 \%
\hline
\( K_m \) & 1 & s\(^{-1}\) & 0.4487 & 8 & 7 \%
\hline
\( K_{H} \) & 1 & s\(^{-1}\) & 0.3 & 8 & 8 \%
\hline
\( \tau_{m} \) & 2 & s & 0.25 & 100 & 80 \%
\hline
\( S_m \) & 2 &  & 0.3 & 8 & 6 \%
\hline
\( V_m \) & 2 & mV & -59.5 & 2 & 1 \%
\hline
\( S_h \) & 2 &  & 0.32 & 30 & 15 \%
\hline
\( V_h \) & 2 & mV & -67.5 & 1 & 1 \%
\hline
\( \gamma_{H1} \) & 2 & s & 1.2 & 20 & 25 \%
\hline
\( \gamma_{H2} \) & 2 & s & 6 & 25 & 25 \%
\hline
\( S_r \) & 2 &  & 0.5 & 100 & 50 \%
\hline
\( V_r \) & 2 & mV & -45.5 & 5 & 5 \%
\hline
\( \lambda_{max} \) & 3 & Hz & 140 & 17 & 2 \%
\hline
\( H \) & 3 &  & 6.8 & 30 & 10 \%
\hline
\end{tabular}
\end{table}

Fig. 2. A: frequency function describing the distribution of peak amplitudes of individual unitary potentials. B: equivalent electrical circuit for a short isopotential segment of a single bundle of circular muscle from guinea pig antrum. \( E_u \) and \( g_u \) represent an equivalent linear background conductance with equilibrium potential equal to the rest potential. \( C_m \) represents the total membrane capacitance of the preparation. \( E_b \) and \( g_b(t) \) represent the total instantaneous unitary conductance modulation with an equilibrium potential equal to the estimated reversal potential for unitary events.
dividing by the mean input resistance gave a value of 36.4 nF for $C_m$.

**METHODS**

**Physiological methods.** The procedures used for the acquisition of physiological data have been approved by the Animal Experimentation Ethics Committee of the University of Melbourne. The procedures are fully described in Suzuki and Hirst (28). Briefly, guinea pigs of either sex were stunned and exsanguinated, and the stomach was removed. The stomach was immersed in physiological saline [in mM]: 120 NaCl, 25 NaHCO$_3$, 1.0 Na$_2$HPO$_4$, 5 KCl, 2 MgCl$_2$, 2.5 CaCl$_2$, and 11.1 glucose bubbled with 95% O$_2$-5% CO$_2$, and cut along the greater curvature, and the mucosa was removed. After removal of the longitudinal muscle layer, circular smooth muscle bundles were visible as discrete clusters of contiguous parallel muscle cells. Tension applied when pinning the preparation showed that the separations between bundles opened in a net-like manner, whereas the muscle bundles maintained their original width. Single bundles of circular muscle (diameter 150–200 μm, length 300–600 μm) were pinned across a glass pipette with 2000 μl of 0.5 M KCl. Use of a low molarity filling solution allowed long-lasting impalements to be obtained. Sometimes, both electrodes were used to measure $E_m$. Alternatively, one electrode was used to pass current and the second, to record $E_m$. Signals were amplified by using an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA), low-pass filtered (cutoff frequency, 100 Hz), digitized, and stored on a computer for later analysis.

Preparations were superfused with physiological saline solution warmed to 35°C and allowed to equilibrate for 2 h before recording began. Nifedipine (1 μM) was routinely added to the physiological saline to prevent involvement of L-type calcium channels in $E_m$ changes.

BAPTA-AM and nifedipine (each obtained from Sigma, St. Louis, MO) were used in these experiments.

**Computational methods.** Simulations involving integration were carried out by using MATLAB 6.0 (The MathWorks, Natick, MA). Stiff differential equation solvers ode15s and ode23s were used. Other simulations were performed by using Daos 7.0 (SciTech, Preston South, Victoria, Australia). Fast Fourier transforms were carried out by using Origin 5.0 (Microcal Software, Northampton, MA). Computations were done on an Intel Pentium 4-based desktop computer.

Seven model parameters (unitary potential time constants 0.434 and 0.077 s, unitary amplitude probability density function $A_p$, equilibrium potentials $-65$ and $-20$ mV, background conductance 227 nS, and the value of 36.8 nF for $C_m$) were individually estimated from the data. One parameter (750 nM for $K_D$) was estimated with reference to the literature (23). Selection of suitable values for the 15 remaining parameters was done by hand optimization of candidate models against 33 experiments that correspond to previously published physiological results [29 experiments shown in Figs. 4–8 of Suzuki and Hirst (28); 4 experiments shown in Figs. 2Aa and 8 of Edwards et al. (6)].

To confirm that the intervals between simulated unitary potentials were in conformity with Poisson statistics, natural logarithm survivor ($\ln($survivor$)$) curves were constructed for periods of simulated membrane noise [method in Hashitani and Edwards (11)]. In each case examined, the $\ln($survivor$)$ plot was well fitted by a straight line indicating that the distribution of intervals was well described by Poisson statistics. Spectral analysis allowed another test on the accuracy of simulated membrane noise characteristics. The spectral densities of simulated membrane noise were estimated by fast Fourier transform and compared with the theoretical curve obtained by direct Fourier transformation of the function defined in Eq. 4 (8). In each case, good agreement was obtained, as is the case for comparative physiologically acquired data. The figures of simulations presented in this paper were each selected from a set of simulated trials, each having the same initial conditions and stimulus but a different sample of the specified unitary conductance modulation distribution.

**Model parameter uncertainties.** Each of the 15 unknown parameters appearing in Eqs. 1–3 was individually increased in value until one or more of the experimental simulations yielded an unsatisfactory simulation. Candidate models were rejected because of failure to produce regenerative responses, spontaneous regenerative potentials occurring at unphysiologically rapid rates, or unacceptable estimates of latency and duration of regenerative potentials. This process was repeated by individually reducing the value of each parameter. Table 1 shows the minimum percent increase and reduction to each parameter that in isolation were sufficient to degrade the simulation capacity of the model to an unacceptable level. These limits indicate the relative uncertainties associated with each parameter. It can be seen that the smallest uncertainties are associated with $V_n$ and $V_h$ that define the voltage window for formation of the intermediate compound.

Because 15 parameters were estimated from fits to a set of 33 experiments, the system of equations was overdetermined. Such redundancy acts to reduce the range of parameter combinations that produce satisfactory simulations.

**RESULTS**

Isolated bundles of circular muscle generate an ongoing discharge of membrane noise (Fig. 3A), which in the absence of stimulation, leads to the spontaneous occurrence of regenerative potentials (Fig. 3C, E). The frequency of these spontaneous regenerative potentials varies from preparation to preparation between ~0.1 and 3 min (28, 31). Figure 3B shows a simulation of 5 min of membrane noise, composed of unitary potentials, generated with a value for $\lambda_{\text{max}}$ (Eq. 3) of 126 Hz. This was 90% of the value chosen as control for $\lambda_{\text{max}}$, and no spontaneous regenerative potentials were generated in the 5-min period shown. Comparable membrane noise recorded from a circular muscle bundle is shown in Fig. 3A. When $\lambda_{\text{max}}$ was set to the control value of 140 Hz, the model generated spontaneous regenerative potentials at an increased rate (Fig. 3D).

Each regenerative potential started with an acceleration of membrane noise followed. Physiologically acquired membrane noise including occasional spontaneous regenerative potentials is shown in Fig. 3C. Increasing $\lambda_{\text{max}}$ to a value of 350 Hz led to the production of regenerative potentials at a rate of ~2 min (Fig. 3F). Figure 3E shows physiological data taken from a circular muscle preparation that generated regenerative potentials at a similar rate.

Regenerative potentials can be initiated by depolarizing the muscle bundles. Characteristically, they be-
Membrane noise is suppressed (6). Figure 4 after each regenerative potential, the discharge of currents of increasing intensity. Figure 4 family of such regenerative responses to depolarizing stimuli are applied (Fig. 5, uli. This is particularly apparent when threshold stimulations of unitary potentials, successive responses are depressed after the regenerative potentials.

Ongoing membrane noise in the baseline regions was reduced after the regenerative potentials. Figure 5 shows simulated responses to 8-nA current pulses of the same duration. The mean latency is shorter, and the variability in latency is reduced. Physiologically acquired comparative data are shown in Fig. 5, A and C, respectively.

Physiological experiments have shown that regenerative potentials can be evoked with a current pulse of short duration (28). In these cases, $E_m$ returns toward its resting value before the regenerative potential starts, suggesting that a period of membrane depolarization can activate a process of messenger formation. To trigger a regenerative potential with a briefer pulse, the intensity of the applied current must be increased (28). Such an inverse correlation between threshold current intensity and pulse duration is accommodated by the present model. Thus the model suggests that brief intense depolarization causes the formation of a threshold level of the intermediate reagent.

Regenerative potentials are also triggered at the break of a period of membrane hyperpolarization. After a period of mild hyperpolarization, the regenerative potential occurs with a long and variable latency. With more profound hyperpolarization, the latency and its variance are reduced until a minimum of ~2.5 s is achieved (28). A family of regenerative potentials recorded from a circular muscle bundle and evoked by

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Because regenerative responses are made up of populations of unitary potentials, successive responses show variation, even when triggered by identical stimuli. This is particularly apparent when threshold stimuli are applied (Fig. 5, A and C). Simulations of three regenerative potentials evoked by a near-threshold depolarizing current of 2 nA for 5 s are shown in Fig. 5B. They show latencies ranging between ~3 and 5 s.

**Fig. 3.** Spontaneous activity. A: membrane noise recorded from an antral circular muscle bundle that generated no spontaneous regenerative potentials over a 5-min period. Resting membrane potential, $-60$ mV. B: simulated membrane noise composed of unitary potentials whose maximum mean frequency of occurrence was 126 Hz (90% of control value for $\lambda_{max}$), C: membrane noise recorded from an antral circular muscle bundle that generated a few spontaneous regenerative potentials over a 5-min period (resting membrane potential, $-60$ mV). D: simulated membrane noise including occasional spontaneous regenerative potentials occurring at fewer than 1/min. The control value of 140 Hz was used for $\lambda_{max}$. E: membrane noise recorded from an antral circular muscle bundle that generated high-frequency spontaneous regenerative potentials (resting membrane potential, $-71$ mV). F: simulated membrane noise including frequent spontaneous regenerative potentials. A value of 350 Hz was used for $\lambda_{max}$. The voltage calibration bar applies to all traces.

**Fig. 4.** Evoked regenerative potentials showing the dependence of latency on stimulus strength. A: regenerative responses evoked by injecting depolarizing currents (5-s duration; 1, 2, 3, 4, and 5 nA; timing shown in C) recorded from an antral circular muscle bundle (resting membrane potential, $-62$ mV). B: simulated regenerative potentials evoked by injecting depolarizing currents of 5-s duration and of 5 intensities (3, 6, 9, 12, and 15 nA; timing shown in C). The input resistance of the bundle was about 3 times higher than that of the model, accounting for the difference in applied current intensity. Note that response latency shortened as stimulus intensity was increased. Voltage calibration bar applies to A and B. C: timing and relative amplitude of injected current pulses.
Characteristically, after each regenerative response is initiated, the discharge of membrane noise falls to a low value. The period during which the discharge of membrane noise is at a low value is associated with a period of depressed excitability. Thus at short intervals, after a regenerative response, the preparation is refractory. As the interval between stimulating pulses is increased, the responses show partial refractory behavior, with full recovery occurring over some 15 to 25 s. Figure 8A shows physiologically acquired data illustrating the recovery of excitability after a conditioning regenerative response. This behavior is replicated by the model, because the voltage-sensitive rate $m_h K_F$ in Eq. 1 recovers from inactivation with a time constant near 6 s at resting $E_m$ ($\tau_h$, Eq. 2 and Fig. 1A). Figure 8B shows that if simulated depolarizing pulses were delivered to the model 5 s apart, the second pulse failed to elicit a response. A 10-s interval allowed sufficient recovery from inactivation for a small regenerative potential to develop. Longer intervals allowed progressively more recovery, and a full amplitude regenerative potential could be evoked after 15–20 s.

When BAPTA-AM (10–20 $\mu$M) was added to the physiological saline for 10–15 min, membrane noise decreased and individual unitary potentials were detected (Fig. 9A) (6). Regenerative responses to applied
depolarizing current (Fig. 9C) and release of hyperpolarizing current (Fig. 9E) could still be evoked, but their amplitudes were reduced to a few millivolts, and individual unitary potentials occurring at a frequency higher than the baseline rate were detected in each response (6). Presumably BAPTA had reduced \([Ca^{2+}]_i\).

It has been shown in hepatocytes that the threshold Ins(1,4,5)P3 concentration for Ins(1,4,5)P3-induced Ca2+ release is inversely dependent on \([Ca^{2+}]_i\) within the store, which in turn depends on \([Ca^{2+}]_i\) (24). Similarly, it was proposed that the transduction process that responds to changes in \([messenger]\) by altering the rate of unitary potential discharge (\(\lambda\)). \(D\): membrane potential, \((E_m)\). \(E\): injected current.

**DISCUSSION**

This article has attempted to provide a mathematical description of the way in which changes in \(E_m\) trigger regenerative responses in single bundles of circular muscle isolated from the gastric antrum of the guinea pig. The model has assumed that when recordings are made from a short bundle of circular muscle, including a small proportion of ICCIM, the bundle can be considered isopotential (6, 28). Experiments employing two microelectrodes have shown that electrotonic degradation of responses to injected current pulses never exceeds 5%, regardless of electrode position and separation (3). Therefore, the actions of the smooth muscle cells within a short bundle are synchronized by close electrical coupling.

An isopotential group of cells can be modeled electrically as a single equivalent cell, even when the component cells have heterogeneous membrane properties. If the cells are sufficiently well coupled so that electrical potential differences between cell interiors are insignificant, then particular channels that arise on only one cell type contribute current that will influence every cell’s \(E_m\). A segment of muscle bundle is then electrically equivalent to a single large cell whose membrane contains all of the membrane channels found on all the smooth muscle cells in the preparation to which are added all the membrane channels found on the ICCIM. The equivalent cell’s surface area is the sum of the surface areas of all the cells, so its membrane capacitance is equal to that of the entire segment of muscle bundle.
When muscle bundles are depolarized, they respond by giving an increased rate of discharge of unitary potentials that together produce a regenerative potential (6). Although not specified by the model, it is assumed that unitary potentials are generated by ICC_{IM} (4, 13). Because the physiological recordings have shown that changes in \( E_m \) initiate a cascade of events that triggers a response after a minimum latency of \( \sim \) 1 s, the first approach was to model the gating process in the way described by Hodgkin and Huxley (15). Assuming a single-step reaction, such delayed responses could only be achieved by dramatically increasing the exponent associated with the activation variable. An alternative approach, which was able to accommodate the physiological observations, was to examine the possibility that a gating mechanism, like that described by Hodgkin and Huxley (15), triggered formation of messenger via a two-step reaction chain. Presumably, this stands in place of a more complex reaction scheme that includes the gated formation of messenger, the release of \( \text{Ca}^{2+} \) from intracellular stores, and the activation of channels, perhaps calcium-activated chloride channels, in the membranes of ICC_{IM} (13). Hence, the state variables precursor and intermediate have not been correlated with identified substances. The equations defining voltage-sensitive behavior are descriptive, in the style of Hodgkin and Huxley’s (15) axonal Na channel activation and inactivation, with their physical parameters being chosen from fits to sets of experimental data.

Implicit in the construction of this schematic model of regenerative potentials are a number of simplifications. First, no attempt has been made to individually model the diffusional, binding, and \( \text{Ca}^{2+} \) release steps that lead from a change in [messenger] to a change in unit discharge rate. These actions presumably take place on a much more rapid time scale than the observed changes in \( E_m \). Therefore, the dose-effect relationship (Eq. 3) was implemented as an instantaneous transfer function. Second, no mechanistic basis is offered for the time course of a unitary conductance modulation. The decay phase proceeds more rapidly than simple exponential dissipation. This could result from \( \text{Ca}^{2+} \) inactivation of \( \text{Ca}^{2+} \) release, or partial inactivation of \( \text{Ins}(1,4,5)P_3 \) receptors by \( \text{Ins}(1,4,5)P_3 \) (22). Such self-inhibition would also be expected to affect the time courses of regenerative potentials. Adding a mechanism of this kind to the model would be expected to improve the fidelity of the decay phases of simulated regenerative potentials.

Frequently, simulated periods of low unitary release rate appear as unrealistically flat baselines (e.g., refractory periods in Fig. 8B). A partial explanation for this may be an inaccuracy in the unitary amplitude histogram shown in Fig. 2A. The first bar in Fig. 2A, which contains the smallest measurable unitary potentials (0–0.5 mV), has 11.5% of the observations. This low value might reflect analytical difficulty in resolving overlapping small events, some of which are lost in noise from other sources. Certainly, increasing the proportion of small events results in simulations that have noisier baseline regions. There is, however, no better empirical measure of small unit frequency in this preparation than the value used.

The linear background conductance used in the equivalent cell membrane model is clearly a simplification. In the experiments described in this paper, L-type \( \text{Ca}^{2+} \) channels were blocked by using nifedipine. However, smooth muscle cells have a number of other voltage and \( \text{Ca}^{2+} \)-sensitive conductances that are presumably activated during a regenerative potential (7). ICC_{MY}, which are not present in antral circular muscle bundles, are likewise reported to have voltage and \( \text{Ca}^{2+} \)-dependent conductances in their membranes (12, 18, 19). Similar conductances may be present in ICC_{IM}, but no definitive measurements have been made from ICC_{IM}.

Many of the voltage-dependent membrane channels inactivate at the depolarized level that is sustained for several seconds during a regenerative potential. T-type \( \text{Ca}^{2+} \) channels generally inactivate rapidly at \( E_m \) positive of \( \sim \) 60 mV (7, 34). Similarly, Koh et al. (20) showed that a voltage-dependent nonselective cation current in murine colonic myocytes inactivated with a
time constant of 86 ms at −45 mV. Delayed rectifier K⁺ channel activation in murine colonic smooth muscle cells is incomplete in the Eₘ range of the regenerative potential. Moreover, the major component of this current inactivates with a time constant at 0 mV and room temperature of 35°C, possibly faster at 1.5 s (21). A-type K⁺ current, which has been described in gastrointestinal tissues, is largely inactivated above approximately −40 mV (1, 32). A window current via this conductance contributes to Eₘ between slow waves in murine antral tissue, but when this current was blocked, changes in spontaneous slow-wave shape were not evident (1). Inward rectifier K⁺ channels are found in canine colonic circular smooth muscle and may be predominantly located in ICC (8), but little inward rectifier current passes at depolarized potentials. In rat fundus, a transient Na⁺ current inactivates within a few milliseconds (33). Furthermore, application of tetrodotoxin has no effect on the mean rate or the variability in rate of generation of slow waves in preparations of guinea pig antrum, so few transient Na⁺ channels may be present (14).

Some other channel types are likely to be present in numbers too small to make a major contribution to regenerative potential shape. Suzuki et al. (29) showed that specific block of large conductance Ca²⁺-activated K⁺ channels with charybdotoxin or of apamin-sensitive Ca²⁺-activated K⁺ channels led to increases of a few millivolts in slow-wave amplitude and increased the probability of spiking. In the present study, the contribution due to these conductances is likely to be even smaller, because of the reduction in the range of [Ca²⁺] i due to nifedipine block of L-type Ca²⁺ channels. Ito et al. (16) showed that glibenclamide caused no detectable change in spontaneous contractions in circular smooth muscle from guinea pig stomach, which suggests that ATP-activated K⁺ channels are not open under normal conditions in this tissue.

It is certain that voltage and Ca²⁺-dependent channels make a contribution to the Eₘ recorded from circular smooth muscle bundles. However, when simulating slow changes within the Eₘ range of the regenerative potential, a linear background conductance provides an adequate approximation for testing the proposed messenger mechanism.

Whereas there is evidence suggesting that the intracellular messenger acting in this process could be Ins(1,4,5)P₃ (14), which furthermore displays voltage dependency in other guinea pig tissue (10), there is no clear idea of what the membrane precursor substance is, or how a change in Eₘ could activate its transformation to an intermediate reagent. Certainly any polar sections in a precursor molecule situated in the cell membrane would be subject to the electrostrictive effects of an electrical charge gradient across the membrane. The molecule need not span the whole membrane for this to be true. Even molecules bound to one side of the membrane will be subject to an electric field due to charge imbalance between the inside and outside of the cell. What proportion of the electric field differential is maintained across the precursor molecule depends on its dielectric constant relative to that of the lipid substrate. The forces that an electric field imposed on polar sections of a precursor molecule or its coreagents could cause a conformal change that catalyzed production of an intermediate form.

It has been tentatively suggested that a G protein might act as part of the voltage-sensing mechanism involved in the initiation of regenerative potentials (13). It has been shown previously that in smooth muscle cells, if Ins(1,4,5)P₃ formation has been initiated by applying the appropriate agonist, its formation can be increased or decreased by depolarizing or hyperpolarizing the preparations (17). In these tissues, whereas a G protein forms part of the stimulatory pathway (2), these observations do not indicate that it is necessarily the G protein that displays voltage sensitivity. However, in bundles of antral muscle, voltage gating can be selectively uncoupled by treating the tissues with N-ethylmaleimide without blocking the resting discharge of unitary potentials (13). Although this agent has widespread actions, one of its properties is its ability to alkylate and, hence, inactivate G proteins (27).

Despite these simplifications, the model developed in this paper allows the replication of many characteristics seen in physiologically acquired data. The responses have a long latency, which is inversely proportional to stimulus strength. Responses to repeated stimuli of constant magnitude show latency jitter, the range of which is related inversely to stimulus strength. Occasional failures of response are observed at low levels of stimulation. The threshold depolarization for evoking a regenerative response is a decreasing function of pulse duration. Regenerative responses can be evoked by the release of applied hyperpolarizing current-anode break excitation. Subsequent to a regenerative response, there is a period of absolute refractoriness followed by a relative refractory period. In control conditions, spontaneous regenerative depolarizations are observed. Such spontaneous transients always begin with a smoothly accelerating pacemaker depolarization that leads into the rising phase and terminate with a more abrupt repolarization. Antral tissue is seen to contract repetitively and fairly regularly. Such oscillations can be modeled by increasing the maximum discharge rate of unitary depolarizations. Responses to depolarizing and hyperpolarizing current injections in the presence of BAPTA-AM can be simulated by reducing the maximum discharge rate of unitary depolarizations. The intervals between unitary potentials can be shown by ln(survivor) plots to have approximately Poisson distributions in both the physiological and simulated cases. The spectral density profiles of membrane noise and its simulation share a characteristic profile, having a knee at a few Hertz and a steep decline, approaching but never exceeding a gradient of −8 at high frequencies.

In summary, the model reproduces a wide range of spontaneous and evoked Eₘ changes characteristic of guinea pig antral circular muscle bundles. Therefore, this study is consistent with the hypothesis that an
intracellular messenger is formed whose production can be modulated by changes in $E_m$ and that the rate of discharge of unitary potentials varies with the concentration of the messenger.

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DISCLOSURES

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REFERENCES