Activation of intestinal smooth muscle cells by interstitial cells of Cajal in simulation studies

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Sperelakis, Nicholas, and Edwin E. Daniel. Activation of intestinal smooth muscle cells by interstitial cells of Cajal in simulation studies. Am J Physiol Gastrointest Liver Physiol 286: G234–G243, 2004; 10.1152/ajpgi.00301.2003.—Activation of a two-dimensional sheet network (5 parallel chains of 5 cells each) of simulated intestinal smooth muscle cells (SMCs) by one interstitial cell of Cajal (ICC) was modeled by PSpice simulation. The network of 25 cells was not interconnected by gap-junction channels; instead, excitation was transmitted by the electric field that develops in the junctional clefts (JC) when the prejunctional membrane fires an action potential (AP). Transverse propagation between the parallel chains occurs similarly. The ICC cell was connected to cell E5 of the network (5th cell of the 5th (E) chain) via a high-resistance junction. The stimulating current, applied to the ICC cell interior, was made to resemble the endogenous undershooting slow wave (SW) of 4 ms) took the ICC cell from a resting potential (RP) of 80 mV to a membrane potential of −41 mV. The slow wave produced a large negative cleft potential in the JC (VJC; ICC-E5). The Vjc brought the postjunctional membrane of E5 to threshold, causing this cell to fire an AP. This, in turn, propagated throughout the SMC network. If the ICC cell was given an RP of −55 mV (like SMC) and a slow wave of 40 mV amplitude (ISW of 1.8 nA), it still activated the SMC network. This was also true when the ICC cell was made excitable (developing an overshooting, fast-rising AP). In summary, one ICC cell displaying a slow wave was capable of activating a network of SMC in the absence of gap junctions.

The voltage generated in the junctional cleft (VJC) is negative and serves to depolarize the postjunctional membrane to its threshold by a patch-clamp-like action (Refs. 8, 13, and unpublished observations). Amplitude of VJC depends on several factors, including the values of RJC and the rate of rise of the AP in the prejunctional membrane.

Simulation shows that propagation had a staircase profile, i.e., propagation velocity in each cell was virtually infinite, coupled with a large junctional delay time (e.g., 0.5 ms) (Refs. 9, 12, and unpublished observations). Thus almost all of the propagation time was consumed at the cell junctions. The EF mechanism and evidence supporting it is summarized in two recent review articles (9, 10). This evidence includes the fact that the intercalated disc contains a high density of fast Na+ channels (4). When 10,000 or 1,000 gap junction channels per junction were placed into the model, propagation velocity became nonphysiologically fast (12).

When several chains of cells were placed in parallel with no gap junction channels between them, excitation was capable of jumping from chain to chain (Ref. 6 and unpublished observations). This transverse transmission was affected by the longitudinal resistance of the interstitial fluid (ISF) between the parallel chains (Rao2). The tighter the packing of the chains in the bundle, the faster the transverse propagation velocity. It appeared that the mechanism for the transverse transmission between the parallel chains was the EF that develops in the narrow ISF space. The moderate hyperexcitability of the basic membrane units composing the cells facilitated successful transfer of excitation by the EF.

In the present study, a two-dimensional planar network of smooth muscle cells (SMCs) consisting of five parallel chains of five cells each (5 × 5 model) was used to investigate activation of the SMC network by a single interstitial cell of Cajal (ICC). It is thought that intramuscular type ICCs (ICC-IM) are the means for physiological activation of the circular smooth muscle layer of the intestine, somewhat analogous to the conduction system of the heart (1–3). Density of ICC-IM varies in the stomach circular muscle but is ~1 ICC cell/25 SMCs in the guinea pig pylorus (14). Gap junction connections occur between the intramuscular ICC-IM cell and an SMC cell (1–3). ICC-IM cells in antrum and pylorus do not initiate slow waves but exhibit regenerative APs in response to slow waves from the ICC-MP (myenteric plexus). We have chosen to model only the passive responses they display after the regenerative component is blocked (1–3). This allows our model to represent, in part, slow waves generated by ICC-MP (1–3). The

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Fig. 1. Diagram of the arrangement of the $5 \times 5$ smooth muscle cell (SMC) planar network with an attached intramuscular-type interstitial cell of Cajal (ICC-IM) making junctional contact with cell E5 of the network. Each cell is represented by four membrane units: one for the junctional membrane at each end of the cell and two for the surface membrane (one up and one inverted). There were no low-resistance connections between cells within each chain (1–5) or between chains (A–E). The radial junctional cleft resistance ($R_{jc}$) was represented by two resistors in parallel (one up and one down). The longitudinal resistance of the interstitial space between the chains ($R_{ol2}$) is depicted. Propagation of simulated action potentials (APs) in this $5 \times 5$ model was examined when the ICC-IM cell either displayed a slow wave (40 mV amplitude) or generated an overshooting action potential (AP).
slow waves are undershooting, long-duration potential changes, having an average amplitude of ~40 mV and an RP of ~60–80 mV (1–3, 14). We have also used an RP of approximately −55 mV for an alternate “depolarized” ICC-IM. In our present PSpice simulation study, it was found that one ICC-IM cell was capable of activating the entire SMC network, regardless of whether gap junctions were present between the ICC-IM cell and the contiguous SMC cell.

METHODS

The details and circuits for PSpice simulation of AP propagation in SMCs were previously given (13). A planar sheet of 25 SMCs [5 parallel chains (A–E) of 5 cells each] was used in the present study. Propagation of APs over the entire network was demonstrated to occur in the absence of gap junction channels (4), so no gap junction channels were used within the SMC network for the present experiments. A single ICC-IM cell was attached to the last cell of the fifth chain (E5 cell) via a special junction (Fig. 1). A small portion of the circuit has been expanded for illustration in Fig. 2. This junction between the ICC cell and E5 was varied by 1) high resistance without gap junction channels and 2) low resistance with a variable number of gap junction channels. A large negative cleft potential (Vjc) developed in this ICC-E5 junction when the ICC cell displayed a slow wave. This cleft potential depolarized the postjunctional membrane of E5 cell to threshold, resulting in excitation of E5 cell, which then propagated an AP that propagated to the other 23 cells of the SMC network.

Because the ICC-IM cell does not exhibit a regenerative AP, the slow wave was simulated by applying a slow-wave current pulse (Isw) to the inside of the ICC cell that would cause its membrane potential to depolarize by 40 mV for a prolonged period (e.g., 4, 8, 16, or 32 ms; standard value, 8 ms). The rate of rise (and rate of fall) of the Isw pulse was 4, 8, 16, or 32 ms (standard value of 4 ms). Amplitude of the slow wave was varied over a wide range (standard value of 40 mV). To produce the standard slow-wave amplitude of 40 mV, the applied Isw was 2.4 nA (ICCA) or 1.8 nA (ICCB). Thus two types of ICC cells, with respect to resting potential (RP), were used 1) RP of −80 mV (ICCA) and 2) RP of −55 mV (ICCB).

In some simulations, a second ICC-IM cell was attached to the SMC network, namely at the last cell of the first chain (A5 cell). Runs were made with simulations of only this second ICC cell, as well as the first ICC cell. In some simulations, the ICC-IM cell was made to generate an overshooting AP (by inserting a GTABLE into the basic units) to determine whether this would be equally effective in activating the SMC network. In the case of the ICCA cell (RP of −80 mV), the AP was fast-rising and the overshoot was to approximately +32 mV (resembling a cardiac AP). In the case of the ICCB cell (RP of −55 mV), the AP was slow-rising and its overshoot was to approximately +11 mV (resembling an AP in an SMC).

Determinations of the total propagation time (TPT) were made by measuring the time difference between when the first response (e.g., AP in E5 cell) reached a membrane potential (Vm) of −20 mV and when the last response (e.g., AP in a cell of the A chain) crossed −20 mV. In some cases, the latest period was measured from the beginning of the Isw pulse applied to the ICC cell to when the AP response in the attached E5 cell crossed −20 mV.

As illustrated in Fig. 1, each cell (SMC and ICC) was represented by four basic units: two units for the surface membrane (one upward and one downward) and one unit for each of the two junctional

**Expanded Circuit of Portion of ICC – Smooth Muscle Cell Network**

![Expanded Circuit of Portion of ICC – Smooth Muscle Cell Network](Image)

**Fig. 2.** Printout of a portion of the circuit used for PSpice simulations of two-dimensional propagation of APs in the 5 × 5 SMC model when activated by a single ICC-IM cell. To allow the circuit elements and labels to be larger, only the circuitry of the ICC-IM cell attached to cell E5 and portion of E4 are shown. In those experiments in which the ICC-IM cell was made excitable, the stimulus (0.5 ms; 0.5 nA) was applied after a 1.0-ms delay) to the inside of the ICC-IM cell, as depicted. In those experiments in which the ICC-IM cell was made inexcitable, a slow wave (40 mV amplitude under standard conditions) was imposed on the cell by applying a slow-wave depolarizing current (Isw) of the appropriate intensity, shape, and duration. Two types of ICC-IM cell were used, with respect to resting potential (RP); 1) RP of −80 mV (ICCA) and 2) RP of −55 mV (ICCB). When the ICC cell was made excitable, the AP in the ICCA cell was fast-rising (ca 300 V/s) and overshot to +32 mV (AP amplitude of 112 mV), and in the ICCB cell was slow rising (ca 15 V/s) and overshot to +11 mV (AP amplitude of 66 mV). Rj, intracellular longitudinal resistance; Rjc, resistance of the junctional cleft; Cj, capacitance of junctional membrane; meg, MΩ; CS, intracellular longitudinal resistance.
membranes. To simplify the recorded traces (APs), voltage markers were placed across only one of the units of each of the 25 cells in the SMC network, namely the upward-facing surface unit. The same was true for the ICC cell. The cleft potential \( V_{JC} \) (measured across \( R_{JC} \)) was measured only for the ICC-E5 junction and the subsequent E5-E4 junction. In addition, the voltage change was measured across the postjunctional membrane (of the E5 cell) at the ICC-E5 junction.

**RESULTS**

As indicated in METHODS, two types of ICC-IM cells were used: ICC\(_A\) and ICC\(_B\). ICC\(_A\) had an RP of \(-80 \text{ mV}\), and when it was allowed to be excitable, its AP was fast rising (maximum rate of change of the command voltage ramp (d\( V \)/dt\(_{max} \)) of \(-300 \text{ V/s}\)) and the overshoot was to \(+32 \text{ mV}\). Thus the AP amplitude was \(112 \text{ mV}\). ICC\(_B\) had an RP of \(-55 \text{ mV}\) (same as the SMC in the \(5 \times 5\) network), and when allowed to be excitable, its AP was slow-rising (d\( V \)/dt\(_{max} \) of \(-15 \text{ V/s}\)) and the overshoot was to \(+11 \text{ mV}\). Thus its AP amplitude was \(66 \text{ mV}\) (Fig. 2).

In some experiments, the ICC-IM cells (ICC\(_A\) and ICC\(_B\)) were made inexcitable (by removing the GTABLE), and instead, a slow wave was generated by internal application of depolarizing current (\( I_{SW} \)). The standard slow-wave amplitude was \(-40 \text{ mV}\). Thus in the ICC\(_A\) cell, \( V_M \) went from \(-80 \text{ mV}\) (RP) to \(-40 \text{ mV}\); in the ICC\(_B\) cell, \( V_M \) went from \(-55 \text{ mV}\) (RP) to \(-15 \text{ mV}\). The standard slow-wave rise time was \(4 \text{ ms}\), plateau of \(8 \text{ ms}\), and fall-time of \(4 \text{ ms}\). Because the SMCs in the network were not given pacemaker properties, the slow-wave plateau duration (and fall-time) were not important.

**Excitable ICC-IM Cells**

When the ICC-IM cells were made excitable, ICC\(_A\) and ICC\(_B\) were capable of activating all 25 SMCs in the \(5 \times 5\) network. This, as illustrated in Fig. 3A, shows a typical result with the ICC\(_A\) cell and Fig. 3B with the ICC\(_B\) cell. The first trace in A and B is the AP of the ICC cell. Figure 3A, right shows the AP at a faster sweep speed.

**Nonexcitable ICC-IM Cells**

Voltage recordings across selected units of cells ICC, E5, E4, and E3. Recordings were made from only a few cells near the ICC-E5 junction to reduce the number of traces and so simplify their identification. This is illustrated in Fig. 4. The circuit block diagram for the ICC cell and SMCs E5, E4, and E3 is illustrated in Fig. 4A. Numbers indicate the points at which voltage was measured corresponding to the traces labeled in B and C. Data from an ICC\(_A\) cell are illustrated in Fig. 4B. Trace 1 is the slow-wave voltage applied to the ICC\(_A\) cell. Trace 2 is the \( V_{JC} \) recorded at the ICC\(_A\)-E5 junction. Trace 4 is the voltage recorded across the postjunctional membrane of cell E5 (\( V_{JU} \); facing the ICC\(_A\)-E5 junction). The three AP traces were recorded from cells E5, E4, and E3. The potential changes of the junctional membranes are superimposed on those for the surface membrane as indicated by the labels. Data from an ICC\(_B\) cell are illustrated in Fig. 4C. Traces are labeled as in Fig. 4B. The main difference is that \( V_{JC} \) (ICC\(_B\)-E5) is smaller in amplitude and shorter in duration. Hence the \( V_{JU} \) trace is similar to other AP traces in cell E5. That is, there is no subtraction from the \( V_{JU} \) trace, as occurs in Fig. 4B.

**Effect of gap junctions.** In these experiments, and all of the following, voltage recordings were made from all 25 cells of the SMC network and from the ICC-IM cell (from the upward-facing surface membrane unit). In addition, a voltage recording was made across \( R_{JC} \) (\( V_{JC} \)) of the last three junctions: ICC-E5, E5-E4, and E4-E3. \( V_{JU} \) of the E5 cell was facing the ICC-E5 junction.

Control response in the absence of gap junctions at the ICC\(_A\)-E5 junction is shown in Fig. 5A for standard conditions. Standard conditions included an \( R_{JC} \) value of 20 M\( \Omega \) throughout the SMC network and a slow wave of 40 mV amplitude (from \(-80 \text{ mV} \) RP to \(-40 \text{ mV}\)) in the ICC\(_A\) cell. The standard slow wave had a rise time of \(4 \text{ ms}\), plateau of \(8 \text{ ms}\), and fall-time of \(4 \text{ ms}\). As can be seen in Fig. 5A, the peak cleft potential (\( V_{JC} \)) at the ICC\(_A\)-E5 junction was \(-10 \text{ mV}\), and it followed the time course of the slow wave. As predicted, the \( V_{JC} \) subtracts from the \( V_{JU} \). \( V_{JC} \) brought the E5 cell to threshold, which then propagated an AP throughout the network.

When a parallel shunt resistance (\( R_{GJ} \)) was inserted across the ICC\(_A\)-E5 junction (from the inside of the ICC\(_A\) cell to the inside of E5 cell), the effect of gap junctions, in parallel with...
the EF mechanism ($V_{JC}$), could be assessed. Figure 5 shows that an $R_{GJ}$ of 1,000 M$\Omega$/H9024, equivalent of one gap junction channel (100 pS each) had almost no effect. However, when $R_{GJ}$ was 10 M$\Omega$/H9024 (equivalent to 1,000 channels) or 1.0 M$\Omega$/H9024 (10,000 channels) cell E5 closely followed the $V_{M}$ change of the ICC A cell, namely the slow wave. The postjunctional membrane of the E5 cell, as expected, also followed the slow wave, but at a reduced amplitude due to

Fig. 5. Effect of adding gap junction channels at the ICC$_A$-E5 junction. A slow wave of 40 mV was imposed on the ICC$_A$ cell (from $-80$ mV to $-40$ mV). A: control with no added gap junction channels ($R_{GJ} = \infty$). B: $R_{GJ} = 1,000$ M$\Omega$ (equivalent to 10 channels). All 25 cells still responded. C: $R_{GJ} = 10$ M$\Omega$ (1,000 channels). Twenty cells were activated. D chain (5 cells) failed to respond, and cell E5 now follows the voltage of the ICC$_A$ cell very closely. Voltage of the postjunctional membrane unit of the E5 cell ($V_{JU}$) also follows the slow-wave voltage of the ICC$_A$ cell but at a reduced amplitude. D: $R_{GJ} = 1.0$ M$\Omega$ (10,000 channels). Twenty cells were again activated. D chain failed to respond, and cell E5 now follows identically the slow-wave voltage of the ICC$_A$ cell.
subtraction of $V_{JC}$. As can be seen, only 20 cells of the SMC network responded in Fig. 5, C and D (failure of one chain). Although cell E5 is excitable, it does not fire an AP because of the “voltage clamping” effect of the slow wave in the ICC cell. Similar results were obtained with the ICC B-type cell (Fig. 6). The control response in the absence of gap junction channels at the ICC-B-E5 junction is shown in Fig. 6 A for standard conditions. The slow wave of the ICC B cell went from $-55$ mV (RP) to $-15$ mV. The peak $V_{JC}$ was approximately $-2$ mV and of short duration. Therefore, the shape of $V_{IJ}$ was nearly identical to the AP across the surface membrane of the E5 cell. $V_{JC}$ was sufficient to excite E5, and an AP was propagated throughout the network.

Fig. 6. Effect of adding gap junction channels at the ICC-B-E5 junction. Slow-wave amplitude of 40 mV (from $-55$ mV to $-15$ mV). A: control with no added gap junction channels. All 25 SMCs were activated. B: $R_GJ = 1.000$ MΩ (equivalent to 10 channels). Twenty-five SMCs responded. C: $R_GJ = 10$ MΩ (1,000 channels). Twenty-two SMCs responded. Cells D3, D4, and D5 failed, and cell E5 now follows the voltage of the ICC B cell identically. $V_{IJ}$ of the E5 cell also follows the slow-wave voltage closely. D: $R_GJ = 1.0$ MΩ (10,000 channels). Twenty-two SMCs were again activated. Cells D3, D4, and D5 again failed, and cell E5 and $V_{IJ}$ identically follow the voltage of the ICC B cell.

Fig. 7. Effect of variation of slow-wave amplitude current (changing $I_{SW}$) in the ICC-A-type cell on the amplitude of junctional cleft potential ($V_{JC}$) at the ICC-E5 junction. The $I_{SW}$ pulse had a standard rise time of 4 ms, plateau of 8 ms, and fall time of 4 ms in all cases. A: $I_{SW} = 4.8$ nA. $V_M$ went from the $-80$ mV RP to $+2$ mV. $V_{JC}$ was $-20$ mV. All 25 SMCs in the network responded. B: $I_{SW} = 2.4$ nA (standard). $V_M$ went from $-80$ mV to $-40$ mV, thus giving a slow-wave amplitude of the standard 40 mV. $V_{JC}$ was $-10$ mV. All 25 SMCs responded. C: $I_{SW} = 1.2$ nA. $V_M$ went from $-80$ mV to $-60$ mV, giving a slow-wave amplitude of 20 mV. $V_{JC}$ was $-6$ mV. All 25 SMCs responded. D: $I_{SW} = 0.6$ nA. $V_M$ went from $-80$ mV to $-70$ mV, giving a slow-wave amplitude of 10 mV. $V_{JC}$ was $-2$ mV. All 25 cells responded.
Therefore, with both ICC_A and ICC_B, insertion of gap junction channels did not facilitate activation of the SMC network. However, TPT was slightly reduced, as would be expected on the basis of one less cell (E5) that needed to be excited and propagated to.

Effect of slow-wave amplitude. Amplitude of the slow wave was varied to determine its effect on the amplitude of the junctional cleft potential ($V_{JC}$) of the ICC-E5 junction. This was done for both the ICC_A-type cell (Fig. 7) and the ICC_B-type cell (Fig. 8). As can be seen in Fig. 7, $V_{JC}$ was $-20$ mV when the slow-wave amplitude was $80$ mV (Fig. 7A), $-10$ mV when the slow wave was the standard $40$ mV (Fig. 7B), $-5$ mV when slow wave was $20$ mV (Fig. 7C), and approximately $-2.5$ mV when slow wave was $10$ mV (Fig. 7D). Thus there was a linear relationship between $V_{JC}$ amplitude and slow-wave amplitude, as shown in Fig. 9A. As can be seen in Fig. 7, the delay time between the beginning of the slow wave and the AP response of the E5 cell (to where $V_M$ crossed $-20$ mV) was slightly shorter; e.g., the delay was $2.7$ ms in Fig. 7A vs. $3.3$ ms in Fig. 7D. The small slow wave, and hence small $V_{JC}$, was capable of activating the E5 cell and propagating throughout the network, because of the high level of excitability (i.e., low threshold) of the SMCs.

As can be seen in Fig. 8, peak $V_{JC}$ amplitude was greater when slow-wave amplitude was greater. However, the relationship between the two parameters was not linear (Fig. 9B). As in the case of ICC_A, delay time between the beginning of the slow wave and the AP response of cell E5 (to $-20$ mV) was slightly shorter; e.g., the delay was $3.1$ ms in Fig. 9A vs. $3.6$ ms in Fig. 9D. There was failure of one (Fig. 9C) or two (Fig. 9D) SMCs at the lower slow-wave amplitudes. On the other hand, TPT (to $-20$ mV) was less with the small slow waves, e.g., $5.3$ ms in Fig. 9A vs. $3.8$ ms in Fig. 9D.

Fig. 8. Effect of variation of slow-wave amplitude in the ICC_A-type cell on the amplitude of $V_{JC}$ at the ICC-E5 junction. The $I_{SW}$ pulse had the standard shape. A: $I_{SW} = 3.6$ nA. $V_M$ went from $-55$ mV (RP) to $+28$ mV. $V_{JC}$ was approximately $-1.0$ mV. All 25 SMCs responded. B: $I_{SW} = 1.8$ nA (standard). $V_M$ went from $-55$ mV to $-14$ mV, thus giving a slow-wave amplitude of $41$ mV (standard). All 25 cells responded. C: $I_{SW} = 0.9$ nA. $V_M$ went from $-55$ mV to $-35$ mV, giving a slow-wave amplitude of $20$ mV. Twenty-four SMCs responded (cell C1 failed). D: $I_{SW} = 0.45$ nA. $V_M$ went from $-55$ mV to $-45$ mV, giving a slow-wave amplitude of $10$ mV. Twenty-three SMCs responded (cells C1 and C2 failed).

Fig. 9. Graphic summary of the effect of variation in the amplitude of the slow wave on the amplitude of the junctional cleft potential ($V_{JC}$) at the junction between ICC cell and E5 cell. A: ICC_A data. B: ICC_B data. $V_{JC}$ amplitude increases nearly linearly with slow-wave amplitude in the case of ICC_A, but the curve flattens in the case of ICC_B. The $V_{JC}$ amplitude is severalfold greater with ICC_A than with ICC_B.
Effect of $R_{JC}$ amplitude. The effect of variation in the amplitude of the radial $R_{JC}$ on amplitude of $V_{JC}$ (ICC-E5 junction) and effectiveness of the ICC$_A$ cell (Fig. 10) and ICC$_B$ cell (Fig. 11) in activating the SMC network was determined. When $R_{JC}$ was varied, it was changed globally throughout the network. As can be seen in Fig. 10, $V_{JC}$ amplitude was $25 \, \text{mV}$ at $R_{JC} = 80 \, \text{M} \Omega$ (Fig. 10A), $17 \, \text{mV}$ at $40 \, \text{M} \Omega$ (Fig. 10B), and $10 \, \text{mV}$ at the standard 20 M$\Omega$ (Fig. 10C), and $6 \, \text{mV}$ at 10 M$\Omega$ (Fig. 10D). There was almost a linear relationship between $V_{JC}$ amplitude and $R_{JC}$ amplitude, as shown in Fig. 12A. All 25 cells of the SMC network responded when $R_{JC}$ was 20 M$\Omega$ (Fig. 12C) or 40 M$\Omega$ (Fig. 12D), but one cell failed to respond at 10 M$\Omega$ (Fig. 12B). Therefore, there is an optimum value of $R_{JC}$.

As can be seen in Fig. 11, $V_{JC}$ amplitude was greater when slow-wave amplitude was greatest. These data are plotted in Fig. 12B. As in the case of ICC$_A$, there was an optimum $R_{JC}$ value for maximum effectiveness of the ICC$_B$ cell in activating the network. All 25 cells responded at $R_{JC}$ of 40 M$\Omega$ (Fig. 12B) and the standard 20 M$\Omega$ (Fig. 12C), but several
cells failed at 10 MΩ (Fig. 12D), and many cells failed at 80 MΩ (Fig. 12A).

DISCUSSION

The present results, using simulated APs, demonstrated that one ICC-IM cell, which displayed a slow wave of 40 mV amplitude, was capable of activating a planar network of 25 SMCs. Successful transmission of excitation occurred in the absence of gap junction channels at the junction between the ICC cell and the last cell (E5) of the SMC network to which it was attached. Addition of many gap junction channels (e.g., 10,000 or 1,000) at that junction simply made the E5 cell follow the V_m change of the ICC cell; the E5 cell then activated the E4 cell whose AP propagated throughout the remainder of the network. If there were only a few gap junction channels (e.g., 1 or 10), then the E5 cell fired an AP after it was brought to its threshold by the cleft potential (V_c) at the junction between it and the ICC cell. Therefore, gap junctions are not required at the ICC-IM junction with an SMC in order for successful activation of the SMC network to occur. This study does not mimic the situation in which voltage-dependent Ca^{2+} channels (VDCC) are blocked, but slow waves still occur (1–3). In future studies, examination needs to be made of networks of SMCs that can respond only passively to slow waves from ICC with or without gap junctions between the SMCs. Furthermore, multiple ICC connected by gap junctions, as in the ICC-MP and connected or not to SMCs by gap junctions, need to be studied.

Two types of ICC-IM cells with respect to the RP were modeled: ICC_A (RP of ~80 mV) and ICC_B (RP of ~55 mV). The latter had an RP similar to that of the SMC in the network. Both types were capable of activating the SMC network, but the first type (ICC_A) worked more readily. This may be due to the fact that the V_c produced at the ICC-E5 junction was greater with the ICC_A cell type (see Fig. 4). Therefore, successful activation of the SMC network should occur whether the RP of the ICC-IM cell is low (e.g., ~55 mV) or high (e.g., ~80 mV).

Even when the ICC-IM cell was made excitable to intrinsically generate an overshooting AP (to ~32 mV in the case of ICC_A and to ~11 mV in the case of ICC_B) by introducing positive feedback between Na^+ or Ca^{2+} conductance and V_m, there was successful activation of the SMC network. This occurred despite the fact that the AP was very fast rising in the case of ICC_A (e.g., dV/dt_{max} of ~200 V/s). In the case of ICC_B, the AP rate of rise was ~10 V/s, i.e., much closer to the rate of rise of the physiological slow wave. Therefore, successful activation of the SMC network also occurred when the ICC-IM cell was made intrinsically excitable. Thus the present results with the special modeling of slow waves are valid.

Special modeling of the slow wave for the ICC-IM cell was done by making it inexcitable (i.e., no positive feedback between Ca^{2+} conductance and V_m) and applying an intracellular stimulating current shaped like a slow wave. This had a number of advantages, including being able to readily vary characteristics of the slow wave such as amplitude, duration, rate of rise, and rate of fall. Because the SMC modeled were not given pacemaker properties (i.e., repetitive firing during prolonged depolarizing pulses), long-duration slow waves were no more effective than those of short duration. Hence, the standard slow wave used had a plateau duration of 8 ms and a rise time of 4 ms. The standard current (I_{sw}) applied to the ICC-IM was 2.4 nA in the case of the ICC_A type and 1.8 nA in the ICC_B type, because those were the current amplitudes required to produce a slow-wave amplitude of 40 mV (about the physiological value) in both types. The slow wave in both types of ICC-IM cell produced substantial negative cleft potentials at the ICC-E5 junction (see Fig. 4).

Circuit analysis of the ICC cell and associated cell junction (ICC-E5) demonstrated that a large negative cleft potential should be generated in the junction, whose magnitude should be nearly directly proportional to the magnitude of the slow wave and I_{sw}. This was actually found for the ICC_A cell. (There was deviation from linearity in the case of the ICC_B cell). As previously reported (6, 13) V_c amplitude was determined by the amplitude of R_{jc} (see Figs. 10 and 11). As shown, there was almost a linear relationship between R_{jc} and the amplitude of V_c in the ICC-E5 junction.

In the present study, there were no gap junction channels between the 25 SMCs of the planar network (5 parallel chains of 5 cells each). It was previously shown (5, 6, 8) that transmission from cell to cell occurs by the EF (negative V_c) generated in the junctional cleft when the prejunctional membrane fires an AP. This accounts for longitudinal propagation. Transverse propagation between parallel chains may occur by a similar EF mechanism, because the longitudinal resistance of the ISF space between the chains (R_{isf}) was a key factor in facilitating the transverse propagation (unpublished observations) (the higher the R_{isf}, reflecting tighter packing of the chains, the better and faster the transverse transmission). Moderate hyperexcitability of the SMCs in the network facilitates the longitudinal and transfer propagation throughout the network by the EF mechanism. The excitability of the basic membrane units was originally adjusted to give a propagation velocity (longitudinal) in the average physiological range with

![Fig. 12. Graphic summary of the effect of variation in the magnitude of R_{jc} on the amplitude of the junctional cleft potential (V_c) at the junction between ICC cell and E5 cell. A: ICC_A data. B: ICC_B data. Note that V_c amplitude increases nearly linearly with magnitude of R_{jc} in both cases. Amplitude of V_c was approximately fourfold greater with ICC_A than with ICC_B.](http://ajpgi.physiology.org/)
the cells having AP characteristics also in the physiological range.

This hyperexcitability may account for why slow waves of smaller amplitude were almost as effective in activating the network in both the ICC_A and ICC_B cases (see Figs. 7 and 8). The same may be true as to why longer rise times for the slow waves were also very effective.

In summary, one ICC-IM cell displaying a simulated slow wave (40 mV amplitude) was able to activate a planar network of 25 SMCs. The ICC-IM cell could be either type A (RP of \(-80 \text{ mV}\)) or type B (RP of \(-55 \text{ mV}\)), but type A was slightly more effective. Successful transmission occurred without any gap junction channels at the ICC-E5 junction and was mediated by the negative junctional cleft potential \((V_{JC})\) developed at that junction. Therefore, gap junctions are not required at this junction. Successful transmission also occurred when the ICC-IM cell was made intrinsically excitable to produce regenerative APs; this was true for both ICC_A and ICC_B types.

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