Properties of a Nonjunctional Current Expressed from a Rat Connexin46 cDNA in *Xenopus* Oocytes

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**ABSTRACT** Connexin46 (cxn46) is a gap junctional protein that was cloned from a rat lens cDNA library. Expression of cxn46 in solitary *Xenopus* oocytes resulted in the development of a large time- and voltage-dependent current that was not observed in noninjected control oocytes or in oocytes injected with mRNA for cxn43 or cxn32. The cxn46-induced current activated at potentials positive to \(-20\) mV. On repolarization to \(-40\) mV, the current deactivated over a period of several seconds. Removal of external calcium caused a marked increase in the amplitude of the cxn46-induced current, shifted the steady-state activation curve to more negative potentials, and altered the kinetics of activation and deactivation. Increasing external calcium had the opposite effect. The ability of cxn46 to induce the formation of cell-to-cell channels was tested in the oocyte pair system. Oocyte pairs injected with cxn46 mRNA + antisense oligonucleotides for *Xenopus* cxn38 were strongly coupled. In contrast, oocyte pairs injected with antisense alone showed no coupling. The inactivation kinetics of the gap junctional channels resembled the deactivation kinetics of the cxn46-induced current in solitary oocytes.

**INTRODUCTION**

Gap junctions are composed of cell-to-cell channels which allow the exchange of ions and other small molecules between neighboring cells. The junctional channels are formed from two protein oligomers called connexons. Each connexon is a hexamer of six protein subunits (connexins) arranged around a central channel pore. A characteristic property of gap junctions is their permeability to large molecules. Fluorescent dye experiments have shown that molecules up to \(\sim 1\) kD can diffuse through mammalian gap junctions (Schwarzman, Wiegandt, Rose, Zimmerman, and Loewenstein, 1981; Imanaga, Kameyama, and Irisawa, 1987). Dyes with extra negative charges had depressed permeabilities compared with less negatively charged dyes of similar molecular mass, suggesting that there are fixed negative charges within the pore (Flagg-Newton, Simpson, and Loewenstein, 1979; Brink and Dewey, 1980).

It is not clear how connexins assemble into cell-to-cell channels. It is conceivable...
that the connexin proteins are inserted randomly into the cell membrane as freely floating connexons or hemichannels and that cell-to-cell channels form when a connexon from one cell comes into contact and pairs with a connexon from a neighboring cell in regions of close plasma membrane apposition. Evidence for the presence of connexin proteins on the nonjunctional plasma membrane comes from a recent study by Musil and Goodenough (1991), who showed that connexin43 is transported to the cell surface before its assembly into gap junctional maculae. If hemi-gap junctional channels exist, there must be a mechanism to prevent them from opening since the presence of open connexons in the nonjunctional plasma membrane would probably lead to cell lysis.

We recently reported the cloning and functional expression of connexin46 (cxn46), a putative gap junctional protein isolated from a rat lens cDNA library (Paul, Ebihara, Takemoto, Swenson, and Goodenough, 1991). Cxn46 has been proposed to be a gap junctional protein on the basis of sequence homology with other members of the gap junctional channel family (see Beyer, Paul, and Goodenough, 1990 and Bennett, Barrio, Bargiello, Spray, Hertzberg, and Saez, 1991 for discussion). In addition, polyclonal antibodies directed against unique peptide segments of cxn46 bind specifically to lens fiber–fiber gap junctions. Expression of cxn46 in Xenopus oocytes results in cellular depolarization and osmotic lysis. Voltage clamp experiments show that these changes are associated with the development of a large time- and voltage-dependent current.

Here we examine the properties of the cxn46-induced current in greater detail. We also show that the expression of cxn46 in pairs of Xenopus oocytes induces the formation of cell-to-cell channels whose inactivation kinetics resemble the deactivation kinetics of the nonjunctional current.

**METHODS**

Rat cxn46, cxn43, and cxn32 mRNAs were prepared as described previously by Swenson, Jordan, Beyer, and Paul (1989). The cDNA clone for cxn46 was a gift from D. A. Goodenough (Harvard University, Boston, MA) and the antisense oligonucleotides were a gift of V. Verselis and T. A. Bargiello (Albert Einstein University, Bronx, NY).

Female Xenopus laevis frogs (NASCO, Fort Atkinson, WI) were anesthetized and a partial ovariectomy was performed. Stage 5 and 6 oocytes were manually defolliculated after a 20-min incubation in Ca²⁺, Mg²⁺-free modified Barth's solution (MBS) and 6 mg/ml collagenase (Worthington Biochemical Corp., Freehold, NJ). Defolliculated oocytes were injected with 40 nl of mRNA for cxn46, cxn43, or cxn32 using a Picospritzer II (General Valve Corp., Fairfield, NJ). The mRNA for cxn46 was diluted to 0.05–0.00005 μg/μl and the mRNAs for cxn43 and cxn32 were diluted to 0.2 μg/μl. The mRNA-injected oocytes were incubated in MBS supplemented with gentamicin (0.05 mg/ml) for 3–24 h at 18°C before recording.

In the cell-to-cell coupling assay, oocytes were initially injected with 40 nl of an antisense oligonucleotide for Xenopus cxn38 (0.5 μg/μl) and allowed to incubate in MBS supplemented with gentamycin at 18°C for 24–48 h in order to eliminate endogenous gap junctional channels (Barrio, Suchyna, Bargiello, Xu, Roginski, Bennett, and Nicholson, 1991). The oocytes were then injected with mRNA for cxn46 or cxn43 and paired following the procedure of Swenson et al. (1989). 0.2–1 mM of cobalt was usually added to the bathing medium in order to stabilize cxn46 channels in the closed configuration and prevent lysis. The oocytes were tested for coupling 14–24 h after pairing.
Electrical Measurement of the Transmembrane Current in Single Oocytes

Transmembrane currents in single oocytes were studied by a two-microelectrode, voltage clamp technique. An Axoclamp 2A (Axon Instruments, Inc., Foster City, CA) was used as the voltage clamp amplifier. The microelectrodes were filled with 0.5–1.5 M KCl, 10 mM EGTA, and 10 mM HEPES (pH 7.4) and had resistances measuring between 0.2 and 1.5 MΩ. The bath electrode consisted of a Ag-AgCl pellet in a 1.5 M KCl agar bridge. Care was taken to reduce the series resistance of the bath electrode to ~1 kΩ. The bath solution contained MBS. All experiments were performed at room temperature (17–20°C). Only oocytes with resting potentials ≤–20 mV, input resistances ≥0.2 MΩ, and cxn46-induced currents ≤5 μA were used in this study. To regulate the level of expression of cxn46, we injected the oocytes with different concentrations of mRNA.

Current records were low pass filtered at 20–100 Hz, digitized, and stored on hard disk for analysis. The pClamp software programs (Axon Instruments, Inc.) were used for data collection and analysis. The time required to change the membrane potential from −40 to 20 mV was ~10 ms. The holding potential was −40 mV except where otherwise noted. At this holding potential, the calcium-activated chloride current would be mostly inactivated (Barish, 1983). No attempt was made to block other endogenous currents since these currents were usually much smaller than cxn46-induced current.

Measurement of Gap Junctional Conductance in Oocyte Pairs

Gap junctional conductance in oocyte pairs was measured using a dual two-microelectrode, voltage clamp technique as described by Spray, Harris, and Bennett (1981). In these experiments, both oocytes were initially voltage clamped to the same holding potential. A voltage clamp step was then applied to oocyte 1, while oocyte 2 was maintained at the holding potential. Under these conditions, the change in current recorded in oocyte 2 would be due entirely to current flowing through gap junctional channels. Gj was determined by dividing Ij by Vj, where Ij = I2 and Vj = V2 − V1. Ij was defined as the current measured 30 ms after pulse onset. Ij was defined as the current at the end of a 24-s voltage clamp step. The normalized steady-state junctional conductance, Gj, was determined by dividing Ij by Ij. The current and voltage traces were monitored continuously throughout the experiment on an oscilloscope and the current traces were digitized and stored on hard disk.

Solutions

MBS contained (mM): 88 NaCl, 1 KCl, 2.4 NaHCO3, 15 HEPES, 0.3 Ca(NO3)2, 0.41 CaCl2, and 0.82 MgSO4, pH 7.4. TEACl MBS was made by replacing NaCl with equimolar TEACl. 0 Ca2+ MBS consisted of MBS with no added calcium. Solutions with different calcium concentrations were made by adding the stated amount of calcium to 0 Ca2+ MBS. Sucrose MBS was made by replacing NaCl with equimolar sucrose. Na methanesulfonate MBS contained (mM): 88 NaOH, 1 KOH, 2.4 NaHCO3, 15 HEPES-Na, 2 gluconic acid-1/2 Ca2+, and 2 gluconic acid-1/2 Mg2+ titrated to pH 7.4 with methanesulfonic acid. Na gluconate MBS contained (mM): 88 Na gluconate, 1 KOH, 2.4 NaHCO3, 15 HEPES-Na, 2 gluconic acid-1/2 Ca2+, and 2 gluconic acid-1/2 Mg2+ adjusted to pH 7.4 with gluconic acid.

RESULTS

Fig. 1 A shows membrane currents recorded from a cxn46 mRNA-injected oocyte in response to a series of depolarizing voltage clamp steps from a holding potential of −40 mV. Expression of cxn46 induced a large time- and voltage-dependent current which will be denoted as Ih. Ih activated on depolarization to potentials more positive
than −10 mV. The time course of activation was slow and the current did not reach a steady-state level during the voltage clamp pulses. After repolarization to −40 mV, \( I_h \) decayed to baseline over a period of several seconds. Fig. 1 C shows the \( I-V \) relation (filled triangles) obtained at the end of the pulse. When similar experiments were performed on noninjected, control oocytes, depolarizing voltage clamp steps usually elicited a mostly passive current response (Fig. 1 B) and the \( I-V \) relation was linear between −40 and 30 mV (Fig. 1 C, filled squares). Expression of cxn43 or cxn32 induced the formation of cell-to-cell channels in oocyte pairs but did not cause any significant changes in transmembrane conductance.

**Selectivity**

Ion substitution experiments were performed to characterize the selectivity of the cxn46 mRNA-induced current for cations and anions. In the initial experiments, the reversal potential of the cxn46-induced current was determined from the reversal of the tail currents. The tail currents were elicited by applying a 24-s depolarizing voltage clamp step to 20 mV and then repolarizing to different potentials. A typical experiment is shown in Fig. 2 A. In modified MBS, the tail currents reversed polarity between −5 and −10 mV in five experiments. Interepisode intervals of several minutes were usually required to allow the cxn46-induced current sufficient time to recover to its original state between pulse episodes.

To determine the reversal potential rapidly and with more precision, a ramp protocol was used (Fig. 2 B). In this protocol, the cell was voltage clamped to 20 mV for 24 s to activate \( I_h \) and then ramped from 20 to −40 mV before \( I_h \) had time to decay to zero. An identical ramp was applied in the absence of a prepulse. The voltage at which the two \( I-V \) curves intersected was taken to be the reversal potential of \( I_h \). The ramp method should measure the reversal potential accurately as long as the difference between the currents in the presence and absence of a prepulse is due to \( I_h \). The validity of this assumption was confirmed by performing similar experiments on noninjected, control oocytes and demonstrating that the difference between the \( I-V \) curves was very small. In MBS, the reversal potential of \( I_h \)
determined by the ramp method was found to be $-9.3 \pm 2.1$ mV in 18 experiments. This value agreed with the reversal potential determined from the reversal of the tail currents. When TEA was substituted for sodium in the external bathing solution, the reversal potential shifted to $-29.2 \pm 1.39$ mV ($n = 9$). Replacement of potassium for sodium ($n = 2$) or gluconate or methanesulfonate for chloride ($n = 9$) had no significant effect on the reversal potential. These findings suggest that $I_h$ is either a nonspecific cation current or a generally nonselective current that cannot discriminate between chloride, gluconate, and methanesulfonate.

**Activation Kinetics**

The kinetics of activation were complex and depended on initial conditions as illustrated in Fig. 3. At a holding potential of $-73$ mV, the time course of activation was very slow and the outward current failed to reach a steady-state level during depolarizing voltage clamp pulses as long as 2 min in duration. Changing the holding potential from $-73$ to $-13$ mV resulted in a marked acceleration of the activation kinetics. At a holding potential of $-13$ mV, the time course of activation was well described by the sum of two exponentials. The time constant of the faster process is plotted as a function of membrane potential in Fig. 5 B (filled triangles). The time course of activation could also be altered by application of a long, depolarizing prepulse as illustrated in Fig. 4 A. In this experiment, two depolarizing voltage clamp steps were applied in succession from a holding potential of $-40$ mV. During the first depolarizing voltage clamp step, the current activated extremely...
slowly. Upon repolarization to \(-40\) mV, the current rapidly decayed to a value close to zero. However, if a second depolarizing pulse to 20 mV was applied 4 s later, the current activated much faster.

The time course of recovery of activation to its original state was studied by a double pulse protocol in which two depolarizing pulses were applied in succession separated by a variable time interval. Typical results are shown in Fig. 4 B. As the interpulse interval grew progressively longer, the amplitude of the outward current at the end of the second pulse decreased and the time course of secondary activation became slower and began to more closely resemble the turn-on kinetics recorded during the initial pulse. Interpulse intervals > 1 min were required for the current to fully recover at \(-40\) mV.

**Kinetics of Deactivation**

Tail currents were recorded on repolarization to different potentials after a depolarizing voltage clamp step to activate the cxn46-induced current. The time constant of deactivation was obtained by fitting the initial decay of the tail current to a single exponential as illustrated in Fig. 5 A. In a few experiments, the time course of deactivation was better described by the sum of two exponentials. The time constant of deactivation was plotted as a function of membrane potential in Fig. 5 B (filled...
squares). The time constant became progressively smaller at more negative potentials.

Effect of Changing External Calcium

When external calcium was removed, the cxn46 mRNA-injected oocytes became extremely leaky and depolarized to $\sim -10$ mV. Voltage clamp experiments demon-

![Figure 4](image-url)

**Figure 4.** (A) Effect of a long, depolarizing prepulse on the time course of activation. $I_h$ recorded in response to two successive depolarizing pulses to 20 mV from a holding potential of $-40$ mV. Dotted line is zero current. (B) Time course of recovery of $I_h$ to its original state after a depolarizing pulse. A 6-s test pulse to 10 mV was applied from a holding potential of $-40$ mV at different time intervals after a 30-s depolarization to 10 mV. The amplitude of $I_h$ at the end of the test pulse was normalized to the amplitude of $I_h$ at the end of the initial pulse and plotted as a function of interpulse interval. The dotted line is the normalized amplitude of the current at 6 s after the onset of the 30-s pulse. (Inset) Superimposed currents for different interpulse intervals. Data in A and B were corrected for a linear leakage component.

![Figure 5](image-url)

**Figure 5.** Time course of deactivation. (A) Tail currents of $I_h$ recorded on repolarization to voltages between $-70$ and 0 mV after a 30-s depolarization to 0 mV. The solid lines were drawn according to the equation $I = A \exp(-t/\tau) + \text{residue}$. (B) Time constants of activation and deactivation plotted as a function of voltage (filled squares for deactivation; filled triangles for activation). Each point represents a mean ± SEM from five experiments. The time constants of activation were obtained by fitting the time course of activation (from a holding potential of $-10$ mV) to the sum of two exponentials. Only the time constant of the faster process is plotted in B.
strate that these changes could be attributed to the effect of calcium on \( I_h \). To prevent the \( \text{cxn46} \)-induced current from becoming excessively large in 0 Ca MBS, the oocytes were injected with very diluted concentrations of \( \text{cxn46} \) mRNA. Fig. 6A shows current records from a \( \text{cxn46} \) mRNA-injected oocyte that was sequentially superfused with solutions containing 0.5, 0.1, or 0 mM added external \( \text{Ca}^{2+} \) and 0.8 mM \( \text{Mg}^{2+} \). Removal of external calcium caused a dramatic increase in the amplitude of \( I_h \) (Fig. 6B). The amplitude of the inward current showed a disproportionate increase in size relative to the outward current. In 0 mM calcium solution, \( I_h \) was visible as a slowly activating inward current at voltages between -50 and -20 mV. The current reversed polarity at -10 mV, a value very close to the reversal potential measured in 0.7 mM external calcium. These changes were usually reversible on washout. Similar changes were not observed in noninjected control oocytes.

Another effect of changing external calcium was to alter the kinetics of activation and deactivation. Reduction of external calcium caused an acceleration in the time course of activation and a prolongation of the tail currents. Fig. 6C shows superimposed tail currents recorded at -60 mV in external solutions containing 0, 0.1, and
0.5 mM Ca^{2+}. The tail currents have been scaled so that the peak amplitude is identical in all three records.

The turn-off kinetics and voltage sensitivity of $I_h$ in 0 calcium solution is shown in greater detail in Fig. 7. Fig. 7A shows currents recorded in response to a series of depolarizing and hyperpolarizing voltage clamp steps to voltages between 20 and -50 mV in 10-mV increments from a holding potential of 0 mV. On hyperpolarization from 0 mV, $I_h$ decayed to a new steady-state level. The initial and steady-state currents are plotted as a function of voltage in Fig. 7B. The initial $I-V$ relation was linear and the reversal potential occurred at -9 mV. The steady-state $I-V$ relation showed a region of negative slope conductance between -50 and -30 mV. The steady-state activation curve was determined by dividing the steady-state current by the initial current as illustrated in Fig. 7C. To compare the steady-state activation

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**Figure 7.** Cxn46-induced currents recorded in 0 calcium MBS. (A) Current was recorded in response to a series of depolarizing and hyperpolarizing voltage clamp steps from a holding potential of 0 mV. The current traces were corrected for a linear leakage component which was estimated by holding the cell at -70 mV and applying a series of hyperpolarizing voltage clamp steps between -70 and -95 mV. Voltage increment, 10 mV. Interepisode interval, 40 s. (B) Initial (filled squares) and steady-state (filled triangles) current plotted as a function of voltage. The initial current was measured 30 ms after pulse onset. The steady-state current was measured at the end of the 24-s step. (C) Steady-state activation curve. The steady-state activation curve was calculated by dividing the steady-state current by the initial current. The solid line was determined from Eq. 1 with $V_o = -27.42$ mV and $A = 0.110$. 

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curve with other voltage-dependent processes, the data were fit by a Boltzmann
equation of the form
\[ \frac{I(V)}{I_\infty} = \frac{1}{1 + \exp \left[ -A(V - V_0) \right]} \] (1)
where \( V_0 \) is the voltage at which the conductance declines to half of its maximum
value and \( A \) is the sensitivity parameter. The parameter, \( A \), can also be expressed as
\( z\frac{q}{kT} \), where \( z \) is the valence of charge \( q \), \( k \) is the Boltzmann constant, and \( T \) is the
absolute temperature. In four experiments \( V_0 = -32.7 \pm 4.9 \text{ mV} \) and \( A = 0.115 \pm
0.016 \) (\( z = 2.9 \)).

The time course of relaxation was fit to a single exponential process as illustrated
in Fig. 8 A. The current relaxation usually deviated from a monoeponential time
course at long times, indicating the presence of a poorly resolved, slow component.
The time constant of relaxation is plotted as a function of voltage in Fig. 8 B. The
potential dependence of the time constant was described by a bell-shaped curve

![Figure 8](https://example.com/figure8.png)

which had a maximum value at approximately -50 mV. There was a large amount of
scatter in the time constants from one experiment to another.

Fig. 9 shows the effect of changing calcium on the steady-state activation curve.
The steady-state activation curve was determined by comparing the initial amplitude
of the tail currents on hyperpolarization to -70 mV after a 24-s voltage clamp step to
different potentials from a holding potential of 0 mV (0.5 mM Ca\(^{2+}\)) or -10 mV (0
Ca\(^{2+}\)). Increasing external Ca\(^{2+}\) from 0 to 0.5 mM produced a 40-mV depolarizing
shift in the midpoint of the activation curve without causing a significant change in
the shape of the curve. It also resulted in a sevenfold reduction in the maximum
conductance. Similar results were obtained in two other experiments.

**Effect of Other Divalent Cations**

To test the specificity of calcium block, the effects of different divalent cations were
compared. Elevation of external calcium or addition of nickel or cobalt to the bathing
solution reduced the amplitude of cxn46-induced current in a dose-dependent manner. This effect developed immediately and was usually fully reversible on washout as illustrated in Fig. 10. Cobalt and nickel were more effective than calcium at blocking I_h. Increasing external Mg^{2+} from 0.8 to 5 mM in the presence of 0.7 mM Ca^{2+} had no effect on I_h. However, in the absence of external calcium, elevating external Mg^{2+} had a blocking effect that was similar but less potent than that of calcium.

**Cxn46 Forms Cell-to-Cell Channels**

The ability of cxn46 to form cell-to-cell channels was tested in pairs of *Xenopus* oocytes. 30 of 35 oocyte pairs injected with cxn46 mRNA and cxn38 antisense were coupled with G_j ranging between 0.24 and 36 μS. In contrast, 18 of 18 oocyte pairs

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**Figure 9.** Effect of calcium on the steady-state activation curve. (A) Currents recorded in the presence of 0.5 mM Ca^{2+} (middle trace) or 0 mM Ca^{2+} (lower trace). Pulse protocol is shown in the upper trace. Data not corrected for leakage. V_h = 10 mV (0.5 mM Ca^{2+}) or -10 mV (0 mM Ca^{2+}). (B) The steady-state activation curve was determined by plotting the initial amplitude of the tail current as a function of prepulse potential (filled squares for 0 mM Ca^{2+}; open circles for 0.5 mM Ca^{2+}). The solid line was the best fit of the experimental data to Eq. 1 with \( A = 0.1187 \) and \( V_0 = -26.8 \) mV for 0 mM Ca^{2+}; \( A = 0.108 \) and \( V_0 = 14.01 \) mV for 0.5 mM Ca^{2+}. (C) Data normalized to the maximum value of the tail current in 0 mM Ca^{2+}.

**Figure 10.** Effects of cobalt on I_h. I_h recorded before, during, and after superfusion with MBS containing 1 mM added cobalt (A) or 1 mM added calcium (B). Voltage clamp steps were applied to 20 mV from a holding potential of -40 mV. Data corrected for leakage.
TABLE I

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of pairs</th>
<th>(G_j) (\mu S \pm SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noninjected</td>
<td>11</td>
<td>0.915 ± 3.04</td>
</tr>
<tr>
<td>Antisense</td>
<td>18</td>
<td>0.0</td>
</tr>
<tr>
<td>46/46+ antisense</td>
<td>35</td>
<td>7.98 ± 8.9</td>
</tr>
</tbody>
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injected with antisense alone showed no detectable coupling. Noninjected controls showed variable but generally low levels of coupling. Table I summarizes the mean \(G_j\) values measured in different types of pairs. Fig. 11A shows a typical family of junctional current traces recorded from a 46/46 pair. The holding potential in this experiment was \(-60\) mV. 1 mM cobalt was added to the recording solution to block \(I_h\). For small transjunctional voltage clamp steps, the junctional current was constant. During larger steps, the junctional current exhibited a slow decay to a new steady-state level. The initial and steady-state \(I-V\) relations are shown in Fig. 11B. The initial \(I-V\) relation (filled squares) was approximately linear over the voltage range between \(-80\) and \(+80\). The steady-state \(I-V\) relation (filled triangles) deviated from the initial \(I-V\) at potentials larger than \(\pm 30\) mV. The normalized \(G_{j-\nu}/V_j\) relation is illustrated in Fig. 11C.

![Figure 11](image-url)

**Figure 11.** (A) Junctional current traces recorded from a 46/46 pair in response to depolarizing and hyperpolarizing transjunctional voltage clamp steps between \(\pm 10\) and \(\pm 80\) mV in 10-mV increments. Holding potential, \(-60\) mV. (B) Initial (filled squares) and steady-state (filled triangles) \(I_j V_j\) relation. (C) Normalized \(G_{j-\nu}/V_j\) relationship. Normalized \(G_{j-\nu}\) was determined by dividing \(I_{\nu}\) by \(I_{\nu}^0\).
DISCUSSION

This study shows that expression of cxn46 induces the formation of cell-to-cell channels in oocyte pairs. In addition, cxn46 mRNA-injected oocytes develop a large, time- and voltage-dependent, transmembrane current called $I_h$. The relationship between the nonjunctional channels and the junctional channels is unclear. There are no structural or biochemical data currently available to indicate whether cxn46 forms nonjunctional channels composed of six subunits or some other oligomeric form.

Properties of $I_h$

The time course of activation depends on initial conditions. It can be dramatically altered by application of a depolarizing prepulse or by changing holding potential. The simplest kinetic scheme that can account for this behavior is a sequential model of channel activation with at least two closed states as depicted below:

$$A \leftrightarrow B \leftrightarrow O$$  \hspace{1cm} (2)

A and B are closed states; O is the open state. In this model, all of the channels reside in A at very negative holding potentials. When the membrane is depolarized above threshold for the first time, the channels go from A to O. Once the channels open, repolarization causes the channels to close to B and then slowly return to A. Depolarized holding potentials also increase the probability that the channel resides in B. The time course of deactivation and the time course of activation at depolarized holding potentials mainly reflect the rapid transition between B and O. The slow time course of activation during the first pulse and the slow recovery of the activation kinetics to their original rate after a depolarizing prepulse reflect the transition between A and B. A similar scheme has been proposed to account for the effect of depolarizing prepulses on endogenous sodium channels in the *Xenopus* oocyte (Baud and Kado, 1984).

Calcium and other divalent cations have a marked effect on $I_h$. In the absence of external calcium, the threshold for activation occurs between -50 and -70 mV. Increasing external calcium reduces the maximum conductance, shifts the steady-state activation curve to more positive potentials, and alters the kinetics of activation and deactivation. These changes cannot be accounted for by pure block. They suggest that in addition to blocking the channel, calcium is screening or binding to negatively charged groups located near the external mouth of the channel. The reduction in maximum conductance, kinetic alterations, and shift in the steady-state activation curve would then be due to a reduction in the local accumulation of sodium and potassium near the mouth of the channel and an alteration in the potential gradient across the channel. Screening of or binding to surface charges has also been postulated to account for the actions of divalent cations on a variety of other ionic channels including the large conductance, calcium-activated potassium channel (MacKinnon, Latorre, and Miller, 1989), the ACh receptor channel (Imoto, Busch, Sakmann, Mishina, Konno, Nakai, Bujo, Mori, Fukuda, and Numa, 1988), and the delayed rectifier in squid giant axons (Perozo and Bezanilla, 1990). Alternatively, calcium may bind to a regulatory site on the channel which affects the voltage sensor.
Gap junctional channels are generally thought to be large, nonselective channels that allow the passage of large anionic dyes such as Lucifer yellow. In this study we demonstrate that \( I_h \) is nonselectively permeable for small monovalent cations such as sodium and potassium. The permeability ratio \( \text{Cl}^-/\text{cation} \) was not determined. However, Paul et al. (1991) reported that \( \text{cxn46} \)-induced channels were permeable to Lucifer yellow, suggesting that the \( \text{cxn46} \)-induced channels pass anions easily.

The physiological significance of \( I_h \) is unclear. Although there have been no reports of a current resembling \( I_h \) in lens fiber cells or cardiac myocytes, it is conceivable that \( I_h \) may have escaped detection because \( \text{cxn46} \)-induced channels are mostly blocked at physiological concentrations of external calcium (i.e., 1.8 mM). Recently, DeVries and Schwartz (1992) reported a hemichannel current in catfish horizontal cells that shows a number of similarities to \( I_h \). It is suppressed by dopamine and is only observed at low external calcium concentrations. Alternatively, \( \text{cxn46} \) may be incorrectly posttranslationally modified in the oocyte leading to the development of open, nonjunctional channels that are not present in the lens. Evidence for this hypothesis comes from the observation that the size of the protein recognized by anti-\( \text{cxn46} \) antisera in immunoblots of whole rat lens is 5–10 kD larger than the translation product in \textit{Xenopus} oocytes (Paul et al., 1991).

\textit{Cxnn46 Forms Gap Junctional Channels}

In a previous study, Paul et al. (1991) found that expression of \( \text{cxn46} \) in \textit{Xenopus} oocyte pairs caused cell lysis. \( \text{Cxnn46} \) pairs could be prevented from lysing by incubation in MBS with 5% ficoll. However, the cells still lost their resting potentials and had such low input resistances that the junctional conductance could not be determined. To avoid this problem, we incubated the \( \text{cxn46} \) pairs in MBS containing 1 mM cobalt, which almost totally blocked the transmembrane conductance changes induced by expression of \( \text{cxn46} \). \( \text{Cxn46} \) pairs incubated in 1 mM Co\( ^{2+} \) consistently demonstrated much higher levels of coupling than noninjected control pairs or control pairs injected with \( \text{cxn38} \) antisense alone. The steady-state \( I-V \) relation tended to be asymmetrical. This asymmetry may be due to incomplete suppression of \( \text{cxn38} \) channels by antisense oligomers or to differences in intracellular calcium or H\( ^+ \) concentrations between the two cells. Alternatively, it may reflect the sensitivity of \( G_j \) to inside-outside potential differences (Obaid, Socolar, and Rose, 1983; Barrio et al., 1991; Verselis, Bennett, and Bargiello, 1991).

It has been previously reported that when hybrid cell-to-cell channels are formed between a \( \text{cxn43} \)-RNA-injected oocyte and a control oocyte expressing endogenous \( \text{cxn38} \) channels, the hybrid channels exhibit a rectifying \( I-V \) relationship (Swenson et al., 1989; Werner, Levine, Rabadan-Diehl, and Gehard, 1989). This behavior was attributed to the gating properties of the \( \text{cxn38} \) hemichannel which appeared to open on hyperpolarization and close on depolarization of the control oocyte. In contrast, we find that \( \text{cxn46} \)-induced channels activate on depolarization and close on repolarization in solitary oocytes. The deactivation kinetics of \( I_h \) in 0 calcium MBS resemble the inactivation kinetics of the gap junctional current, suggesting that the gating properties of \( I_h \) may account for the voltage- and time-dependent behavior of the gap junctional channels.
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