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Measurement of Single Channel Currents from Cardiac Gap Junctions

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Cardiac gap junctions consist of arrays of integral membrane proteins joined across the intercellular cleft at points of cell-to-cell contact. These junctional proteins are thought to form pores through which ions can diffuse from cytosol to cytosol. By monitoring whole-cell currents in pairs of embryonic heart cells with two independent patch-clamp circuits, the properties of single gap junction channels have been investigated. These channels had a conductance of about 165 picosiemens and underwent spontaneous openings and closings that were independent of voltage. Channel activity and macroscopic junctional conductance were both decreased by the uncoupling agent 1-octanol.

CELL-TO-CELL COMMUNICATION IS mediated by gap junctions in many tissues (*1*). Studies of the passive electrical properties of atrial and ventricular trabeculae (*2*), nodal tissue (*3*), and Purkinje fibers (*4*) have shown that all parts of the heart form an electrical syncytium of coupled cells. In all of these studies, indirect evidence has been used to argue that gap junctions mediate the electrotonic coupling. Evidence has included measurements of the diffusion of intracellular ions and tracer molecules from cell to cell (*5*), and ultrastructural analysis showing that the specialized membrane structures between cardiac fibers resemble the permeable junctions of other tissues (*6*). The macroscopic conductance of gap junctions has been determined in pairs of adult heart cells (*7–9*). However, it has not heretofore been possible to record the activity of putative gap junction channels between myocardial cells, or to measure their individual conductance or kinetic properties.

Spray *et al.* (*10*) introduced the use of two separate voltage-clamp circuits to measure directly the junctional conductance between pairs of amphibian blastomeres. A similar approach has been applied to pairs of adult mammalian heart cells (*7–9*) isolated by standard methods (*11*). However, in these experiments, the activity of single gap junction channels could not be measured because the signal-to-noise ratio in such cell pairs is quite low. If gap junction channels have an estimated resistance of 10 Gohm (*12*), only 4 pA of current would flow through an open channel between a pair of cells with a 40-mV transjunctional potential (V_j). Metzger and Weingart (*8*) found that a pair of adult ventricular cells typically had an input resistance (R_m) of about 35 Mohm. About 1200 pA of holding current would be needed to produce the required 40-mV difference in the membrane potential (V_m) between the members of such a cell pair. That is, the opening of a single junctional channel would result in an increase over the background current of about 0.3%, an amount that would be lost in the current noise. In contrast, small spheroidal cells such as those from embryonic heart and other tissues have input resistances in the gigaohm range (*13, 14*). With patch-clamp electrodes in the whole-cell recording configuration (*15*) holding current are only a few picoamperes. Against this background, a 4-pA junctional channel current is easily recognized. Using pairs of rat lacrimal gland cells, Neyton and Trautmann (*14*) measured the conductance of junctional channels but the single channel events were not apparent. With heart cell pairs, we have recorded quantal changes in junctional conductance on the order of 165 pS that correspond to the opening and closing of single gap junction channels.

Dissociation of the ventricles of 7-day chick embryos by techniques previously described (*16*) yielded a suspension of single cells containing a small percentage of cell pairs and clusters. Pairs comprised two sin-

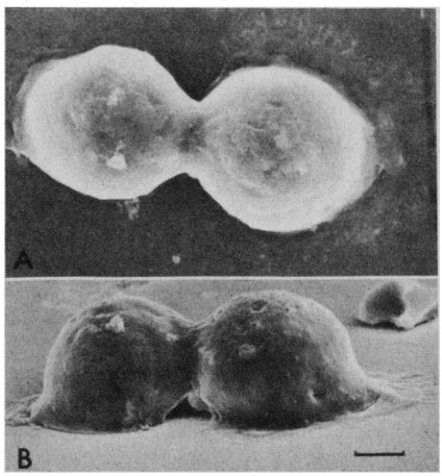

Fig. 1. Scanning electron micrographs of a pair of 7-day chick ventricle cells typical of those used in our study. (A) Top view. (B) Cells tilted 70° from vertical. Scale = 2 μm. After dissociation in a solution containing crystalline trypsin (50 μg/ml), deoxyribonuclease (5.5 μg/ml), and bovine serum albumin (fraction V, fatty acid poor, 1 mg/ml), cells were washed in medium and incubated for 24 hours in vitro in a humidified gassed atmosphere of 85% N_2, 10% O_2, and 5% CO_2. The culture medium contained (by volume) 25% M199, 2% heat-inactivated horse serum, 4% fetal bovine serum, 67.5% potassium-free Ham's F-12, 1% L-glutamine, 0.5% penicillin-G, and 4.3 m*M* KCl. Cells adhering to a chip of polylysine-coated plastic (Thermonox) were washed five times in 37°C balanced salt solution (BSS) containing 142 m*M* NaCl, 2.5 m*M* KCl, 0.8 m*M* $MgSO_4$, 0.9 m*M* NaH_2PO_4, 1.8 m*M* $CaCl_2$, 5.5 m*M* dextrose, and 10 m*M* Hepes (*p*H 7.4). They were fixed in 1.0% glutaraldehyde in BSS, stained in 1% osmium tetroxide, dehydrated from ethanol to Freon-113 by the exchange method (*25*), and critical-point dried. This treatment produced a shrinkage of about 30%. After a 5-nm layer of gold palladium was deposited on the cell surface, the cells were photographed in an ISI Model DS-130 scanning electron microscope fitted with a LAB-6 lanthanum hexaboride electron emitter. (Courtesy of R. Apkarian, SEM Laboratory, Yerkes Regional Primate Research Center, Emory University.)

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gle cells, each 12 to 18 μm in diameter (Fig. 1) with nonjunctional membrane resistances (R_m) of 2 to 5 Gohm and input capacitances of 5 to 8 pF. The pathways for current flow with the double whole-cell recording configuration are illustrated in Fig. 2A. To measure macroscopic junctional conductance (G_j), the two cells were simultaneously

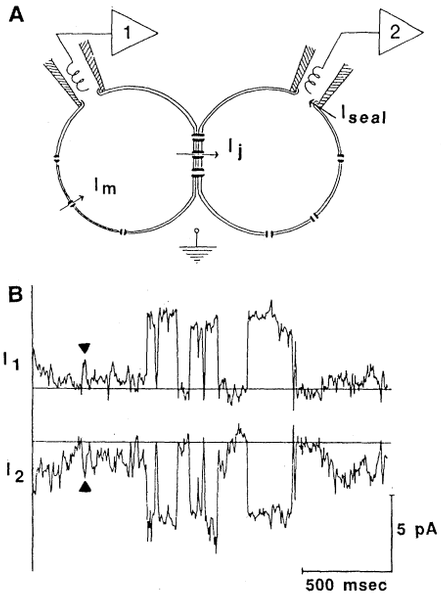

Fig. 2. Measurement of junctional and nonjunctional currents. (A) A diagram of the two-cell preparation, showing the different pathways for current flow. Two current pathways, through the membrane (I_m) and through the seal (I_{seal}), exist between the cell interior and external ground. A change in either of these conductances in one or the other cell results in a change in holding current for that cell, but has little or no effect on the current measured in the opposite cell (assuming that the access resistance between the electrode and cell interior is much lower than R_j). In contrast, a current between the two cells (I_j) resulting from an increase in G_j is recorded simultaneously as inward into the more negative cell, and outward from the more positive cell. (B) Junctional membrane current (I_1 and I_2) in a cell pair with $V_1 = -40$ mV, $V_2 = -80$ mV, and $V_j = (V_2 - V_1) = -40$ mV. The spontaneous current fluctuations of equal magnitude and opposite polarity in the two cells result from changes in G_j. The five distinct quantal events are presumed to result from a single junctional channel opening repeatedly. The conductance of this channel is 125 pS. Smaller conductance changes of about 50 pS were also seen (arrows). The baseline currents reflect a G_j of 1.54 nS, which is equivalent to an average of 12 open gap junction channels of this size. The filter frequency for this and other current records was 100 Hz. Cells were bathed in BSS. The patch electrode solution contained 125 m*M* KCl, 1.0 m*M* KH_2PO_4, the disodium salt of adenosine triphosphate (3.0 m*M*), the disodium salt of phosphocreatine (3.0 m*M*), 0.1 m*M* EGTA, and 10 m*M* Hepes (*p*H 7.1). Separate patch-clamp amplifiers were used to obtain gigaseals (5 to 50 Gohm) on each member of the cell pair and the membrane patches were broken to achieve the whole-cell recording configuration. The access resistance was 10 to 20 Mohm.

clamped through a sequence of 5-mV, 300-msec voltage steps from −80 to +80 mV to record the background current of each cell (I_1 and I_2) with V_j equal to 0 mV. A second voltage sequence was then applied with the voltage of cell 1 (V_1) stepping from −90 to +70 mV and the voltage of cell 2 (V_2) from −80 to +80 mV, thus imposing a transjunctional voltage gradient ($V_j = V_2 - V_1$) of +10 mV at each step. The transjunctional current (I_j) was determined as the difference in I_2 between the first ($V_j = 0$) and second ($V_j = 10$ mV) voltage sequence. Thus any voltage-activated transmembrane currents had been subtracted from the I_j measurement. The G_j was calculated as I_j/V_j.

The junctional conductance between *Xenopus* blastula cells increased in quantal steps immediately after pairing (*17*), suggestive of the sequential appearance of unitary low-resistance pathways. From this result, and by analogy with nonjunctional membrane channels (*18*), we expected that single gap junction channels might exhibit brief openings that would be detected as rectangular, quantal increases in junctional current at constant V_j. However, with two cells clamped at different holding potentials in the configuration shown (Fig. 2A), changes in holding current in one or the other electrode could result from current pathways opening in either the nonjunctional or junctional membrane. These two situations were readily distinguishable. An increase in transmembrane current through nonjunctional channels or through the seal in one cell would be recorded mainly in the electrode attached to that cell, with only a negligible current being seen through the opposite electrode (the magnitude of this transjunctional current would be determined by the ratio of the transjunctional resistance (R_j) to the access resistance between the electrode and cell interior). In contrast, a change in junctional conductance would be recorded as a flow of current into the cell held at a more negative potential and out of the more positive cell. That is, nonjunctional current would normally be seen only by one electrode of the pair, while junctional conductance changes are measured by the two electrodes as simultaneous signals of equal magnitude and opposite sign.

Two cells were clamped at constant V_m and V_j for several minutes ($V_1 = -40$ mV, $V_2 = -80$ mV, $V_j = V_2 - V_1 = -40$ mV) (Fig. 2B). With the procedure in Fig. 2A, G_j was 1.54 nS. The rectangular equal and opposite conductance changes are indicative of the opening and closing of single junctional channels. Records of continuous channel activity from four pairs of cells, containing 124 open-close events, yielded a

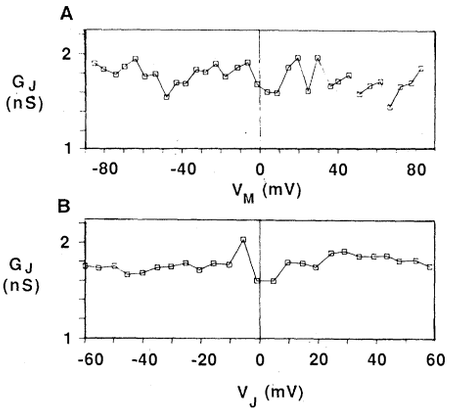

Fig. 3. Voltage-dependence of G_j. (A) Dependence of G_j on V_m. After measuring background currents (I_1 and I_2) over the range −80 to +80 mV in both cells, with $V_j = 0$, V_1 was stepped from −90 to +70 mV and V_2 from −80 to +80 mV in 5-mV, 300-msec increments. The V_j was thus held constant at +10 mV. The I_j was determined as the difference in I_2 between the two voltage sequences. The G_j was calculated at each step as I_j/V_j. (B) Dependence of G_j on V_j. V_1 was held at −40 mV while V_2 was stepped from −100 mV to +20 mV, to vary V_j from −60 to +60 mV. In both (A) and (B), the I_j values were averaged for two voltage sequences.

mean open time of 332 ± 459 (SD) msec, a mean closed time of 1740 ± 4767 msec, and a probability of being in the open state of 0.16. Open times ranged from 16 msec to 4 seconds. These are much longer open times than those of voltage-activated potassium channels in the chick heart cell membrane (*19*), and more closely resemble those of the high-conductance calcium-activated potassium channels in skeletal muscle and other cells (*20*). The long open and closed periods of the latter channels were interrupted by bursts of flickering. Such flickering, if it exists, would not be seen here because the 100-Hz low-pass filtering of our signals would exclude events shorter than a few milliseconds duration.

Average junctional channel conductance was 164 ± 26 (SD) pS. The channel conductance was predicted to be 100 pS from studies of junctional permeability to tracer molecules of different sizes in insect salivary gland cells (*12*) and 120 pS in pairs of rat lacrimal gland cells (*14*). Many of our records also had smaller events, usually of briefer duration, that seemed to represent an additional junctional conductance of about 50 pS (Fig. 2B, arrows). These events, identified by the equal and opposite signals in the two channels, may represent a subconductance state of the gap junction channel and may be analogous to the subconductance states of the acetylcholine receptor channel (*21*), which are postulated to result from conformational changes in the channel macromolecule (*22*).

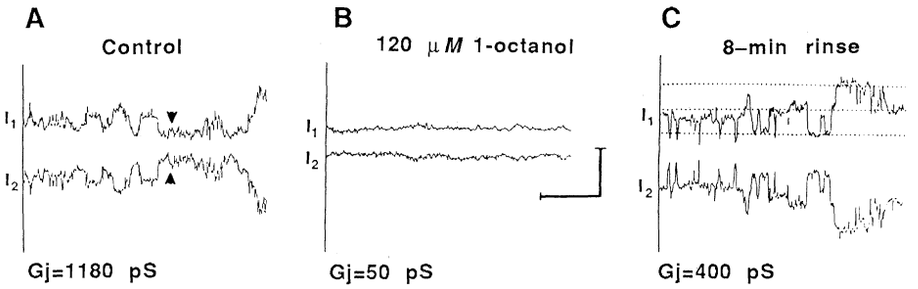

Fig. 4. Suppression of junctional channel activity and blockade of G_j by 1-octanol. Each panel contains a representative 4-second segment of a record from the same pair of cells with $V_j = -40$ mV ($V_1 = -40$ mV, $V_2 = -80$ mV; traces filtered at 100 Hz). In these experiments, G_j was measured periodically during each recording period by applying brief 10-mV depolarizing pulses to cell 1 from a holding potential of −80 mV, while V_2 was held constant at −80 mV. Net I_j in response to the resultant V_j of −10 mV was measured as I_2; $G_j = I_j/V_j$. (A) Control records in BSS 2 minutes after both membrane patches were disrupted for whole-cell recording, showing frequent unitary channel events and occasional 50-pS conductances (arrows). G_j = 1180 pS. (B) Records from the same cell pair, 3 minutes after perfusion with 120-μ*M* 1-octanol. The I_j was 0 pA in some pulses and about 0.5 pA in others, corresponding to a transiently open conductance of about 50 pS. (C) Tracings from the same cell pair after 8 minutes of bath perfusion with BSS (1 ml per minute, bath volume = 2 ml) to show recovery of gap junction channel activity and of macroscopic conductance. The dotted lines correspond to two discrete conductance levels of 260 pS. At this time, macroscopic G_j had recovered to 400 pS. Vertical scale in (B), 20 pA or 500 pS; time scale, 1 second; these apply to all three panels.

In some tissues, G_j is voltage-dependent. Junctional conductance varies with V_m in *Chironomus* salivary gland cells (*23*) and with V_j in fish and amphibian embryo blastomeres (*24*). In contrast, measurements in adult rat and guinea pig ventricular cells (*7–9*) indicate that cardiac gap junctions are voltage-independent. We have examined the dependence of G_j on V_m and V_j in embryonic heart cell pairs. The G_j, measured when V_m ranged from −80 to +80 mV in the presence of a constant V_j of 10 mV, showed no dependence on V_m (Fig. 3A). To determine the dependence of G_j on V_j, V_1 was held constant at −40 mV while V_2 was clamped through a sequence of 10-mV steps from −100 to +20 mV, to vary V_j from −60 to +60 mV. The G_j was also independent of the transjunctional voltage gradient (Fig. 3B). Similar relations were found in six other cell pairs.

If G_j between heart cells depends upon the opening of individual junctional channels, then treatments that reduce G_j should suppress individual junctional channel activity. The uncoupling agent, 1-octanol, reversibly blocks G_j in at least three cell systems including adult heart cells (*9*). Macroscopic G_j was measured in pairs of cells before applying 1-octanol, and channel activity was then recorded continuously for several minutes with V_j at −40 mV. In control buffer, beginning 2 minutes after achieving access to the interiors of both cells, G_j was 1180 pS. During this period, spontaneous channel activity included numerous single channel events (Fig. 4A), and occasional multiple channel openings. Single channel conductance was 188 ± 33 pS. Within 3 minutes after addition of 1-octanol to the bath, the two cells became electrically decoupled, as indicated by the decline of macroscopic G_j to less than 50 pS. During this blockade of G_j, activity of the 188-pS gap junction channels was eliminated (Fig. 4B). However, in such records, many 50- to 70-pS events, like those identified in Figs. 2B and 4A, could still be seen. Superfusion with control solution at 1 ml per minute produced a gradual reversal of the macroscopic decoupling, and a reappearance of single channel activity. After 8 minutes of perfusion, G_j had increased to 400 pS and channel activity resembled that in the control records (Fig. 4C). After 17 minutes of superfusion with buffer, G_j returned to 850 pS, which was 72% of the initial value.

Recently, Neyton and Trautmann (*14*) measured the single channel conductance of gap junctions in paired lacrimal gland cells from rat. They found the channels to have a conductance of 120 pS, only a weak ion selectivity, and no voltage dependence. Lower conductance states were also present in their preparation, but there are some interesting differences between their results and our own. First, they reported that the transitions between the open and closed states of the channel were slow, with exponential time constants of 70 to 90 msec. Our current records show that the transition between the open and closed states occur rapidly in most cases, at least ten times faster than the values that Neyton and Trautmann reported. Second, the channels present in the lacrimal gland cells were not observed to open and close spontaneously in the time course of the voltage protocols used. These workers applied V_j gradients lasting only 500 msec to their cell pairs, whereas we obtained our channel recordings by maintaining a constant V_j of −40 mV for a period of several minutes at a time. Since most of the channel openings that we observed lasted for a few hundred milliseconds, these events might easily have been missed with voltage steps of only half a second.

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