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Letter to the Editor

Do Connexin Channels Have a Residual Conductance State?

A question has arisen recently as to whether gap junction channels possess a low conductance state that is distinct from conventional channel subcondutance states by virtue of its rare and kinetically slow transitions between the closed state and the "residual" state of the gap junction channel (Bukauskas and Weingart, 1993, 1994; Bukauskas et al., 1995; Weingart and Bukauskas, 1993). This so-called residual conductance state was first observed in single channel recordings obtained in insect cell pairs undergoing de novo gap junction formation following initial cell-to-cell contact (Bukauskas and Weingart, 1993; Weingart and Bukauskas, 1993). Characterized by a slow transition (≥10 ms) to a low conductance state from which rapid transitions (≤1 ms) to a fully open channel and conventional subconductance states occur, and the absence or rare closure to the original closed state of the channel, again with long transition times, Bukauskas et al. (1995) report in the June 1995 issue of the *Biophysical Journal* that the residual conductance state is also observed with mammalian connexins transfected into a mammalian (HeLa) cell line in de novo or preformed cell pairs.

This latest observation is in contrast with single channel recordings of human Cx37 channels expressed in mouse N2A cells and cell-attached or inside-out patch recordings from a different invertebrate gap junctional membrane, the septum of the earthworm median giant axon (Veenstra et al., 1994; Ramanan and Brink, 1993). These two distinct single channel recordings were obtained from preformed gap junctions, and both gap junction channels exhibit subconductance states that gate infrequently to the closed (nonconducting) state of the channel. Initially, we attributed the observation of the residual conductance state to different experimental conditions and cell types, more specifically to de novo formation of cell pairs by mechanical manipulation. The contention that a residual conductance state is an inherent property of most gap junction channels including mammalian connexin channels, as illustrated for murine Cx40, is in apparent contradiction with results from our laboratories with several avian and mammalian connexins, including rat Cx40, expressed in a different communication-deficient (mouse N2A) cell line. Fig. 1 illustrates examples of chick Cx42, rat Cx40, and chick and rat Cx43 single channel closures to the closed state, as evidenced by the return to the identical current baseline in the postjunctional (nonpulsed) cell obtained before the transjunctional voltage (V_j) pulse. Furthermore, these transitions to the "ground" state occur with peak-to-peak transition times of ≤8 ms, which is twice the minimum event duration for a full amplitude event in our recordings with a 100-Hz filtering and 2-kHz digital sample rate (recording dead time = 2 ms). In most cases, 1-kHz recordings digitized at 10 kHz illustrate that these open- or substate-to-closed-state transitions occur in the 100- to 500-μs range, which is again near the temporal limit of our recordings (Veenstra et al., 1994; Ramanan and Brink, 1993). Fig. 2 illustrates three transitions between the main state and closed state of the rat Cx43 channel (Fig. 1 *A*) at both 100-Hz and 1-kHz settings. In all cases, the transition times decreased by a factor of 5 or greater, thus indicating that the transition rates are being limited by the response time of the filter. Owing to the temporal limitations of the recording instrumentation, extreme caution should be taken when attempting to assign distinct conductance or gating behavior of a gap junction channel based on point-to-point analysis of event transitions as performed elsewhere (Chen and DeHaan, 1992; Bukauskas et al., 1995).

Analysis of single gap junction channel currents in preformed pairs is complicated by the possibility of a main state of a second channel remaining silent while existing in a subconductance state. Combining this "silent" channel with the gating of the apparent single channel between the observed main-sub-closed states can result in channel recordings that bear a characteristic resemblance to the closed-residual-main-state records described elsewhere. This is best illustrated by recordings we obtained from a Cx37 cell pair where main-state gating was observed frequently for only one channel during an entire 2-min recording at V_j = -30 mV with two stable low conductance states, one that gated frequently between the main state and substate and one that gated infrequently between either the substate or the ground state (Fig. 3). However, there was only one brief opening to a second main state, which implies that two channels were active, whereby the sum of the two produced a recording where one substate was evident 98% of the recording time (Fig. 2, *A–C*). This explanation is feasible given the published records of single channel activity demonstrating the presence of one long-lived substate of the 300-pS main-state Cx37 channel (Veenstra et al., 1994). In these single and two channel records, rapid transition times (limited by the dead time of the recording instrumentation) between all three conductance states were evident for all events. This point is best illustrated by the display (Fig. 3, *C* and *D*) of all four event transitions between the first stable substate and the ground state at the routine 100-Hz and rapid 1-kHz low pass filtration rates. Most of these 100-Hz transitions fall within the same 8-ms duration as noted for the rat Cx43

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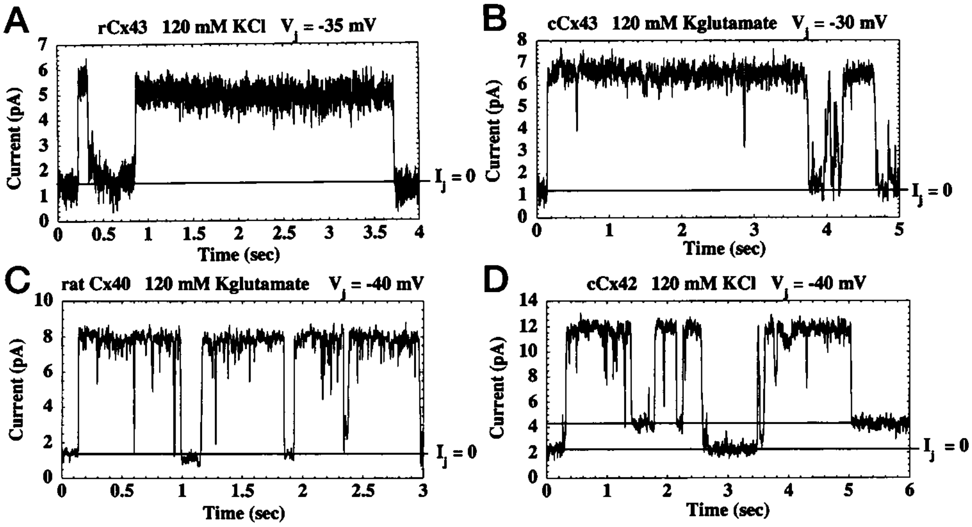

FIGURE 1 : Examples of initial junctional currents in response to the onset of a transjunctional voltage (V_j) pulse for rat Cx43 (*A*), chicken Cx43 (*B*), rat Cx40 (*C*), and chicken Cx42 (*D*). The first 100 ms of each current record indicates the current baseline (ground state, $I_j = 0$ pA) before (and after) the onset of the V_j pulse. The duration of each V_j pulse to the indicated value was 2 min. Only the whole cell current from the postjunctional (nonpulsed) cell is shown to illustrate the initial openings and closings of each connexin channel between the main (open)-state and a closed-state equivalent to the zero junctional current level ($I_j = 0$). The ground state is not equivalent to 0 pA for the whole cell patch clamp owing to a small holding current (1–2 pA in all cases) applied to the cell to maintain the desired holding potential. All analog recordings were low-pass filtered at 100 Hz and digitally sampled at 2 kHz. All cells exhibited a small holding current of 1–2 pA required to maintain the holding potential constant at 0 mV. Transitions between the channel main state and ground state are clearly evident for each connexin channel with rapid kinetics ($\leq$8 ms). A subconductance state is also observed for chicken Cx42, and transitions between this substate and the ground state were infrequent ($n = 38$). There were 5, 155, 75, and 15 channel openings for the main state of the rat Cx43, chicken Cx43, rat Cx40, and chicken Cx42 channels, respectively.

channel with one exception. Again, at the higher 1-kHz filter rate, all event transitions are a factor of 2–5 times faster than at 100 Hz and clearly $\leq$6 s in duration. Furthermore, within these transitions, 500- to 1700-μs intervals are observed, which account for $\geq$70% of the event amplitude. In conclusion, we do not observe any difference in the kinetics of event transitions between the main state or substate and closed (ground) state of the connexin channels we have analyzed, nor can we make any distinction between a substate and a residual conductance based on the characteristics attributed to this stable low conductance state by Bukauskas et al. (1995).

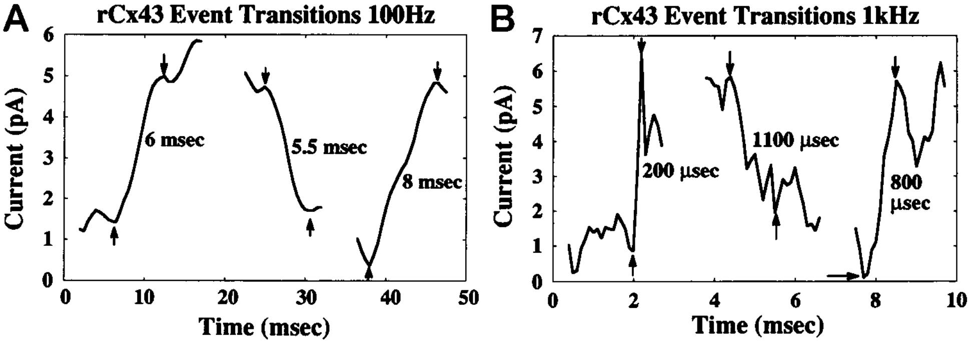

FIGURE 2 Event transitions of the rat Cx43 channel. *A*, Three transitions between the main state and ground state of the same rat Cx43 channel shown in Fig. 1 *A* are displayed at higher temporal resolution at the 100-Hz low-pass filter, 2-kHz digital sample rate settings. All full amplitude event transitions had durations of $\leq$8 ms. *B*, The same three transitions after being low-pass filtered at 1 kHz and resampled at 10 kHz. All event transitions occur 5 to 30 times faster at the higher sampling rate, which demonstrates that the transition times are being limited by the dead time of the of the recording instrumentation.

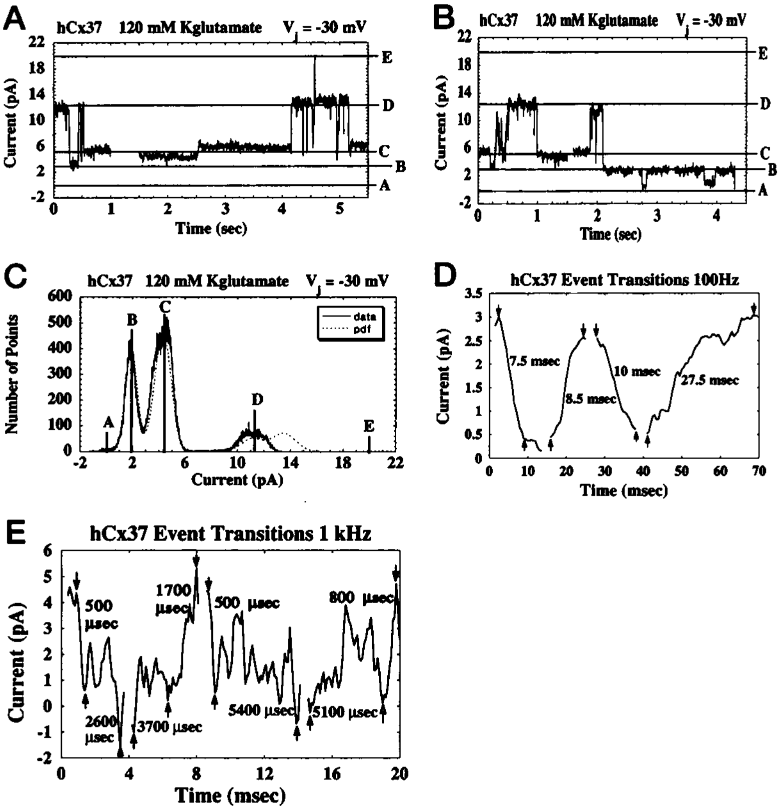

FIGURE 3 Human Cx37 channel activity depicting a low conductance with a high open probability (0.98, level B) with substate and main-state activity of a Cx37 channel superimposed upon this current baseline. (*A*) Onset of the -30-mV V_j pulse and channel activity observed 14 s later (gap of 13 s in record) demonstrating the lone opening of a second Cx37 channel. Transitions between all conductance states except the ground state (*A*),are observed to have short transition times (≤2 ms). (*B*) Continuation of the same -30-mV V_j pulse depicting transitions to the ground state. These four time intervals occurred 23, 43, 60, and 63.25 s after the onset of the pulse. (*C*) Current amplitude histogram (*solid line*) and probability density function (pdf) (*dashed line*) of the first 60 s of the -30-mV pulse. From the pdf fit assuming one main-state and two independent low conductance channels, the probabilities for each state are (level *A* through *E*, respectively) 0.005, 0.289, 0.583, 0.122, and 0.017. Event counts (*A-B*, *B-C*, *C-D*, and *D-E*, respectively) were 21, 143, 37, and 1. Note that the calculated pdf predicts additional channel activity not evident in the recording, which is indicative that the lower conductance states are substates of the Cx37 channel. (*D*) Four transitions between levels *A* and *B* (sub- and ground states) are displayed at higher temporal resolution to illustrate the duration of each event transition under routine recording conditions (100-Hz analog, 2-kHz digital). Note that only one transition has a duration ≥20 ms. (*E*) The same transitions as in panel *D* displayed at higher time resolution (100-Hz analog, 2-kHz digital). The full amplitude transition times measured between the solid arrowheads are displayed in solid type and the minimum transition intervals, which account for ≥70% of the event amplitude, are given in the outlined type. Again, the transition rates are a minimum of 2–5 times faster for all events at the higher sample rate. There is no clear kinetic distinction between these transitions between the closed and subconductance states of the human Cx37 channel, or between the closed and main states of the rat Cx43 channel.

REFERENCES

Bukauskas, F. F., C. Elfgang, K. Willecke, and R. Weingart. 1995. Biophysical properties of gap junction channels formed by mouse connexin40 in induced pairs of transfected human HeLa cells. *Biophys. J.* 68:2289–2298.

Bukauskas, F. F., and R. Weingart. 1993. Multiple conductance states of newly formed single gap junction channels between insect cells. *Pflügers Arch. Eur. J. Physiol.* 423:152–154.

Bukauskas, F. F., and R. Weingart. 1994. Voltage-dependent gating of single gap junction channels in an insect cell line. *Biophys. J.* 67:613–625.

Chen, Y.-H., and R. L. DeHaan. 1992. Multiple-channel conductance states and voltage regulation of embryonic chick cardiac gap junctions. *J. Membr. Biol.* 127:95–111.

Ramanan, S. V., and P. R. Brink. 1993. Multichannel recordings from membranes which contain gap junctions. II. Substates and conductance shifts. *Biophys. J.* 65:1387–1395.

Veenstra, R. D., H.-Z. Wang, E. C. Beyer, S. V. Ramanan, and P. R. Brink. 1994. Connexin37 forms high conductance gap junction channels with subconductance state activity and selective dye and ionic permeabilities. *Biophys. J.* 66:1915–1928.

Weingart, R., and F. F. Bukauskas. 1993. Gap junction channels of insect cells exhibit a residual conductance. *Pflügers Arch. Eur. J. Physiol.* 424:192–194.

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