Two Distinct Gating Mechanisms in Gap Junction Channels: CO₂-Sensitive and Voltage-Sensitive

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ABSTRACT The chemical gating of single-gap junction channels was studied by the dual whole-cell voltage-clamp method in HeLa cells transfected with connexin43 (HeLa43) and in fibroblasts from sciatic nerves. Junctional current (I_j), single-channel conductance, and I_j kinetics were studied in cell pairs during CO_2 uncoupling and recoupling at small transjunctional voltages $(V_j < 35$ mV: V_j gating absent) and at high V_j $(V_j > 40$ mV: V_j gating strongly activated). In the absence of V_j gating, CO_2 exclusively caused I_j slow transitions from open to closed channel states (mean transition time: ~10 ms), corresponding to a single-channel conductance of ~120 pS. At $V_j > 40$ mV, V_j gating induced fast I_j flickering between open, γ_j(main state), and residual, γ_j(residual), states (transition time: ~2 ms). The ratio γ_j(main state)/γ_j(residual) was ~4–5. No obvious correlation between I_j fast flickering and CO_2 treatment was noticed. At high V_j, in addition to slow I_j transitions between open and closed states, CO_2 induced slow transitions between residual and closed states. During recoupling, each channel reopened by a slow transition (mean transition time: ~10 ms) from closed to open state (rarely from closed to residual state). Fast I_j flickering between open and residual states followed. The data are in agreement with the hypothesis that gap junction channels possess two gating mechanisms, and indicate that CO_2 induces channel gating exclusively by the slow gating mechanism.

INTRODUCTION

Direct cell-to-cell communication is mediated by gap junction channels made of two hemichannels (connexons). Each connexon is composed of six connexins (Cx) radially arranged around the pore. Treatments known to alter the cytosolic $[Ca^{2+}]_i$ or $[H^+]_i$ induce channel closure (chemical gating), resulting in cell uncoupling (Bennett et al., 1991; Beyer, 1993; Peracchia et al., 1994). Gap junction channels are also sensitive to transjunctional voltage (V_j) (reviewed in Bennett and Verselis, 1992) and transmembrane potential (V_m) (Obaid et al., 1983; Verselis et al., 1991). Both chemical and voltage-gating sensitivities vary among connexins.

When transjunctional voltage (V_j) gradients are applied to poorly coupled cells, the single-channel current usually flickers between two or more levels. Over the years it has been assumed that the channels flicker between a channel open state, γ_j(main state), and a closed state. However, recent studies on cell-to-cell channel formation in insects and mammalian cells have demonstrated that newly formed channels subjected to V_j gradients primarily operate between open and residual conductance states, γ_j(residual state), a state in which γ_j is 20–25% of that of the open channel (Weingart and Bukauskas, 1993; Bukauskas et al., 1995). This suggested that the V_j gating mechanism closes the channel only partially. These studies also showed that during coupling formation the first channel opens slowly to

γ_j(main state), whereas the subsequent transitions between γ_j(main state) and γ_j(residual state) are rapid. A slow kinetics of I_j transitions was also observed during channel gating by membrane (inside-out) potential (V_m) and lipophilic agents, and was believed to reflect the behavior of a second, V_m-sensitive gate distinct from the V_j-sensitive gate (Bukauskas and Weingart, 1994; Bukauskas et al., 1995).

The present study has tested the hypothesis that gap junction channels possess two distinct gating mechanisms: 1) one that is V_j-sensitive, with fast kinetics (~2 ms) of junctional current (I_j) transitions, and exhibiting a residual state; and 2) one that is CO_2-sensitive or, more generally, a chemical gating mechanism, with slow kinetics (~10 ms) of I_j transitions. Evidence for the existence of fast and slow I_j transitions was previously reported in insect (Bukauskas and Weingart, 1994) and HeLa-40 (Bukauskas et al., 1995) cells. The data were gathered from rat fibroblasts cultured from sciatic nerves and from HeLa cells transfected with Cx43 (HeLa-43); fibroblasts, like HeLa-43 cells, primarily express Cx43 (Crow et al., 1990; Beyer et al., 1989). A preliminary account of these data has been published (Bukauskas and Peracchia, 1996).

MATERIALS AND METHODS

Fibroblasts were freshly isolated from the sciatic nerves of neonatal rats (Brockes et al., 1979). HeLa-43 cells were stably transfected with Cx43 (Willecke et al., 1991; Traub et al., 1994). Cells were grown in Dulbecco's minimum essential medium containing 10% fetal calf serum. The cells were seeded at a density of about 10^4 cells/cm² onto sterile coverslips placed in multiwell culture dishes. Electrical measurements were performed 1–3 days after plating. The coverslips were transferred to an experimental chamber mounted on the stage of an inverted microscope equipped with phase-contrast optics. The chamber was perfused with a modified Krebs-Ringer solution (in mM): NaCl, 140; KCl, 4; CaCl₂, 2; MgCl₂, 1; glucose, 5; pyruvate, 2; HEPES, 5 (pH 7.4). Patch pipettes were

filled with saline containing (in mM): KCl, 130; NaCl, 10; MgATP, 3; MgCl$_2$, 1; CaCl$_2$, 1; EGTA, 10 (pCa ~8); HEPES, 5 (pH 7.2). The electrical properties of single-gap junction channels (Veenstra and De-Haan, 1986) were studied by the dual whole-cell patch clamp (Spray et al., 1981; Neyton and Trautmann, 1985). After the establishment of whole-cell patch clamps with tight seal, the membrane potentials (V_1, V_2) were clamped, and the associated currents (I_1, I_2) were recorded individually in each cell of the pair. Deflections in I_1 and I_2, coincident in time and opposite in polarity, correspond to changes in I_j.

The electrical behavior of individual gap junction channels can be monitored by dual whole-cell patch clamp when cell coupling is minimal, such as in poorly coupled cell pairs, during coupling formation (Chow and Young, 1987; Churchill et al., 1993; Weingart and Bukauskas, 1993), or in the final stages of uncoupling factors. In the present investigation cell uncoupling was induced by bubbling the perfusion solution with 100% CO$_2$.

The conductance of gap junction channels is determined as $g_j = I_j/(V_2 - V_1)$. The state of $I_j = 0$ (all channels in closed state) was ascertained by superimposing on the basic holding potential of cell 2 a 50-mV pulse (100 ms), and by witnessing the absence of an associated current of opposite polarity in cell 1. Signals were recorded on videotape (Digital Data Recorder VR-100; Instrutech Corporation). For analysis, signals were filtered at 5 kHz (8-pole Bessel; -3 dB) and digitized at 1 kHz with an A/D converter (TL-1; Axon Instruments). Data acquisition and analysis were performed with Pclamp software (Axon Instruments) and Sigma Plot (Jandel Corporation).

RESULTS AND DISCUSSION

In cell pairs monitored by dual whole-cell patch clamp, exposure to 100% CO$_2$ caused a reversible drop in junctional conductance (g_j) down to zero in ~30 s (Fig. 1). With only a few channels remaining in operation, single-channel activity could be monitored both just before complete uncoupling (Fig. 1 A and B, *left traces*) and at the beginning of recoupling (Fig. 1 A and B, *right traces*). Recoupling usually occurred 5–10 min after CO$_2$ washout.

The single-channel behavior differed, depending on V_j. With 100% CO$_2$, in cells subjected to a V_j of 30 mV (Fig. 1 A), each channel closed by undergoing a single ~120-pS γ_j transition of slow kinetics from open to closed states (Fig. 1 A, *left trace*). The transition time of the last channel closing was about 7 ms (Fig. 1 A, *inset a*). To demonstrate the kinetics of I_j transitions, some experimental records are displayed with sampling points at 1-ms intervals (Fig. 1 *insets*, and Figs. 2–4, *insets*). The state of zero I_j (all channels in closed state) was ascertained by superimposing on the holding potential of cell 2 (V_2) a positive pulse (50 mV, 100 ms) and by witnessing the absence of an associated current in cell 1. During recoupling, the first channel reopened with a transition time of ~10 ms (Fig. 1 A, *right trace* and *inset b*). Subsequent channels reopened with similar slow transition times (6–20 ms). The selective activation of only the CO$_2$-sensitive gating mechanism is possible at a relatively low V_j because, as reported in many studies, Cx43 is poorly sensitive to V_j gating (Veenstra et al., 1992; Moreno et al., 1992; Miyoshi et al., 1996). The V_j value of 30 mV was chosen to compromise between the need to avoid V_j gating and the need for sufficient resolution for single-channel current recording.
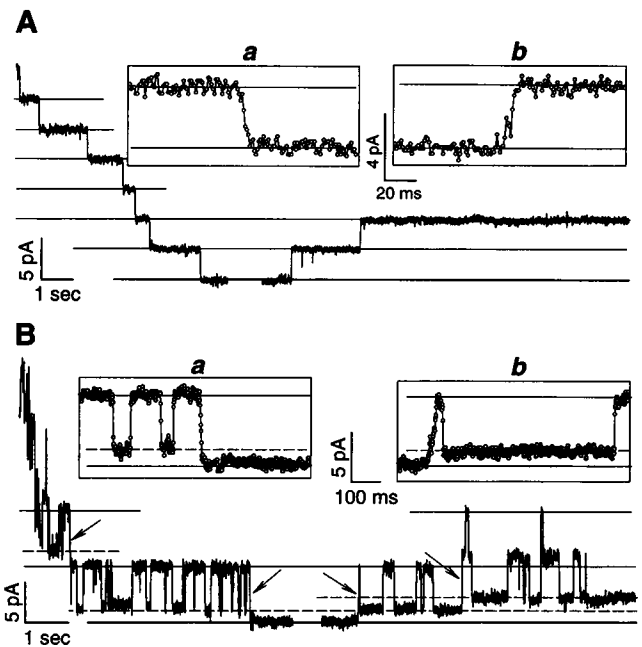

FIGURE 1 Effects of CO$_2$ on single-channel gating in gap junctions of fibroblast cell pairs subjected to V_j of 30 (A) or 55 (B) mV. At either V_j, exposure to 100% CO$_2$ causes a reversible drop in g_j down to zero in ~30 s. With only a few channels operating, the gating behavior of individual channels was monitored just before uncoupling (A and B, *left traces*) and at the beginning of recoupling (A and B, *right traces*). With a V_j of 30 mV (A), each channel closes by a single junctional current (I_j) transition of about 120 pS from the open, γ_j(main state), to the closed state (A, *left trace*), and reopens in 5–10 min with a transition from the closed state to γ_j(main state) (A, *right trace*). The signals in the insets were sampled at 1-ms intervals and plotted with an extended time scale. They illustrate the I_j transition time during the last channel closing, ~7 ms (A, *a*), and first channel opening, ~8 ms (A, *b*). With a V_j of 55 mV (B), the channels exhibit two types of I_j transition: 1) between open and closed states (~120 pS), with a transition time of ~10 ms (B, *left trace, arrows*), and 2) between open and residual states (~90 pS), with a transition time of ~2 ms. The channels eventually reopen, during CO$_2$ washout, by first undergoing an I_j transition of slow kinetics from the closed state to the open state (B, *right trace, arrows*), followed by fast flickering between the open and residual states. Note that during recoupling, when two operating channels are both in the residual state, g_j equals the sum of two γ_j(residual state) conductances (B, *dashed lines*), indicating that each channel is partially closed to 20–25% of its full (open channel) conductance. The last channel closes with a transition time of ~10 ms (B, *a*), and the first channel opens with a transition time of ~19 ms (B, *b*). The channel gating behavior at V_j of 30 and 55 mV indicates that under the influence of CO$_2$, the CO$_2$-sensitive gating mechanism closes the channel completely. During recoupling, each channel first opens slowly to γ_j(main state), indicating that in a chemically gated channel the V_j-sensitive gate is open.

With a V_j gradient of 55 mV (Fig. 1 B), uncoupling and recoupling differed from those observed at a V_j of 30 mV. I_j flickering was more frequent and could be differentiated in two types according to amplitude and transition kinetics: 1) I_j transitions of ~120 pS conductance with a transition time of 8–25 ms (Fig. 1 B, *arrows*), and 2) I_j transitions of ~90 pS with a transition time of 1–3 ms (Fig. 1 B). Characteristic of the former type are the slow I_j transitions: ~10 ms for the last channel closing (Fig. 1 B, *inset a*) and ~19
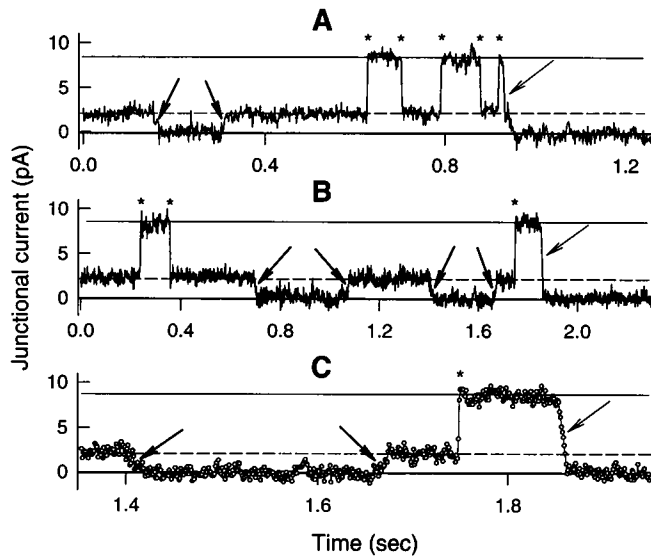
FIGURE 2   Two typical records of I_j transitions (A and B) monitored from a HeLa-43 cell pair during the last channel closing events induced by CO_2 at a V_j of 70 mV. Three types of I_j transition are observed: 1) slow transitions from open to closed states (*thin arrows*), 2) fast transitions between open and residual states (*asterisks*), and 3) slow transitions between residual and closed states (*thick arrows*). (C) A portion of the trace shown in B (*right half*) plotted with an extended time scale. The distribution of the sampling points along the trace (C, *open circles*; sampling interval: 1 ms) demonstrates the difference between fast and slow I_j transitions. The levels of closed and open conductance states are indicated by continuous lines, and those of residual states by dashed lines. All of the transitions were confirmed as junctional by witnessing synchronous current transitions identical in amplitude but opposite in polarity in current records monitored from the other cell of the pair (data not shown).
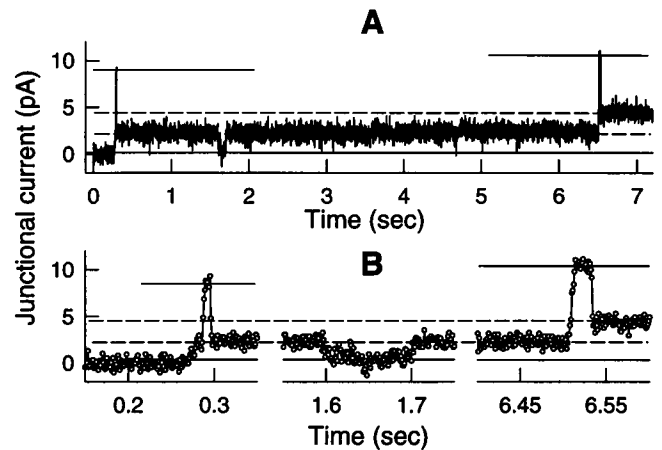

FIGURE 3   Typical example of a long I_j recording from a HeLa-43 cell pair during CO_2 washout (A). With a V_j of 75 mV, recovering channels first open briefly to the fully open state and then remain primarily at the residual state. Subsequent recovering channels follow the same pattern. Note that when two channels are in operation, their combined residual conductance is equal to twice the γ_j(residual state). Three regions of trace A are shown in trace B with an extended time scale: first channel reopening (*left*), spontaneous transition between residual and closed states (*middle*), and second channel opening (*right*). The distribution of the sampling points along trace B (*open circles*; sampling interval: 1 ms) demonstrates the difference between fast and slow I_j transitions. The levels of closed and open γ_j states are indicated by continuous lines, and those of residual γ_j states by dashed lines.

ms for the first channel opening (Fig. 1 B, *inset b*). All of the fast flickering transitions were between open and residual states, and the slow transitions were between open and closed states. Whereas γ_j transitions of ~120 pS and slow kinetics were observed at V_j of both 30 and 55 mV, fast transitions of ~90 pS were only observed at V_j higher than 40 mV.

These data suggested that CO_2 induces uncoupling by activating a slow gating mechanism that functions independently of the V_j gating mechanism. To test this idea, we have studied the effect of CO_2 on cell pairs subjected to even higher V_j (70 mV and greater). At these V_j, I_j exhibited frequent flickering, characterized by short periods at open state and long periods at residual state (low open channel probability). Figs. 2 and 3 show I_j records of cells exposed to CO_2 at V_j of 70 mV during the uncoupling (Fig. 2) and 75 mV during recoupling (Fig. 3). At these V_j, I_j transitions with slow kinetics were observed not only between open and closed states (Fig. 2, *arrows*), but also between residual and closed states (Fig. 2, *thick arrows*), the latter with amplitudes of ~30 pS. This indicates that the CO_2-sensitive gate can operate independently of the voltage gate, being able to close the channel completely either when the voltage gate is open (~120 pS γ_j transitions) or when it is closed (~30 pS γ_j transitions). The transition time of I_j flickering

between residual and closed states was in the range of 5–20 ms, similar to that between open and closed states. Because of the small amplitude of these transitions, a more precise evaluation was hard to perform. Transitions of fast kinetics between open and residual states were observed frequently (Fig. 2, *asterisks*). During CO_2 washout, recovering channels opened briefly at first to a full open state (Fig. 3 A and B, *left and right traces*) and then remained primarily at residual state (Fig. 3 A). Spontaneous transitions between residual and closed states were also observed (Fig. 3 B, *middle trace*). Similar spontaneous transitions between open and closed or residual and closed states with slow kinetics have also been observed in poorly coupled cell pairs (Bukauskas and Peracchia, unpublished observations) and in experiments on new channel formation (Bukauskas and Weingart, unpublished observations on insect and HeLa cells), but at relatively low frequency (approximate range 0–0.1 Hz). In control conditions, at the single-channel level, relatively small or moderate V_j initiates fast I_j transitions between open and residual states. High V_j shows multiple substates exhibiting a conductance intermediate between open and residual conductance states (Bukauskas and Weingart, 1994; Bukauskas et al., 1995).

Recently some questions have been raised about the existence of a residual conductance state in gap junction channels (Veenstra and Brink, 1996). We believe that our present data on CO_2 uncoupling confirm previous evidence for the existence of a residual conductance, at the single-channel level, and a dual gating mechanism (Bukauskas and Weingart, 1994; Bukauskas et al., 1995).

Kinetic analysis was performed to describe in detail the slow I_j transitions observed with CO_2. Examples of both smooth and fluctuating transitions from open to closed and from closed to open states are shown in Fig. 4 A and B, respectively. Fluctuating transitions (Fig. 4 A and B, *bottom traces*) may be indicative of a gating process involving a cooperative or sequential participation of individual connexins. Fig. 4 (C and D) shows averaged traces of I_j transitions from open to close and from closed to open states, respectively. Averaging was performed by synchronizing individual I_j records at the midtransition point (*vertical lines*). Fittings of the averaged data with a sigmoidal function (Fig. 4, C and D, *continuous lines*) show that the I_j transitions have a time course with a mean transition time of ~10 ms.

Individual I_j transition values were measured as the transition time between 90% and 10% of γ_j(main state). Fig. 5 (*upper and middle plots*) shows slow transition kinetics data, averaged from five fibroblast and six HeLa-43 experiments and presented as frequency histograms of transition times for closing and opening events, respectively; data from both cell types were pooled together, as there was no significant difference between them. Note that on average
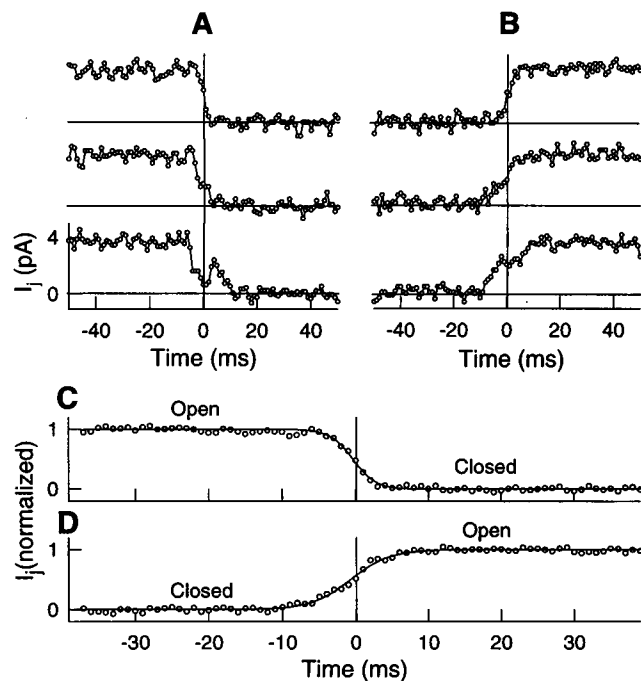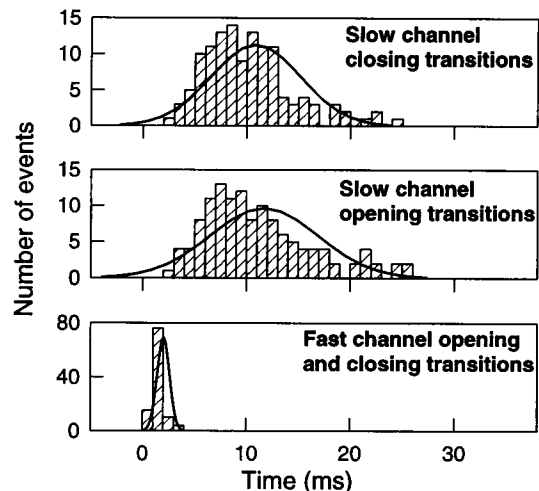

FIGURE 5 Frequency histograms of I_j transition time from open to closed states (*upper plot*), from closed to open states (*middle plot*), and between open and residual states (*bottom plot*). Data were collected from fibroblasts and HeLa-43 cells at V_js ranging from 25 to 75 mV. Data for the upper and middle plots were obtained during uncoupling and recoupling periods, respectively, and were calculated as the transition time between 90% and 10% of γ_j(main state). The bottom panel shows a frequency histogram of fast I_j transitions (open to residual and residual to open states). Continuous lines show Gaussian curves with mean values of 10 ± 0.8 ms ($n = 124$), 11 ± 0.9 ms ($n = 127$), and 2.0 ± 0.2 ms ($n = 105$) for the upper, middle, and bottom plots, respectively. There was no statistically significant difference for slow I_j transitions observed during uncoupling and recoupling periods.

FIGURE 4 Details of slow I_j transitions monitored from fibroblast cell pairs subjected to a V_j of 30 mV during CO_2-induced uncoupling (A and C) and recoupling (B and D). (A and B) Examples of I_j transitions from open to closed states (*continuous line*) and from closed to open states, respectively. (C) Average of nine individual records similar to those shown in A. Before averaging, all of the records were synchronized at their midtransition point (*vertical lines*). Transitions displaying significant fluctuations (A, *bottom trace*) were not used for averaging. (D) Average of 10 individual records similar to those shown in B. Fittings of the averaged curves with a sigmoidal function (C and D, *continuous lines*) show that the I_j transitions have a time course with a mean transition time of ~10 ms.

the CO_2-sensitive gating mechanism closes and opens the channel with I_j transition times of 10 ± 0.8 ms ($n = 124$) and 11 ± 0.9 ms ($n = 127$), respectively (Fig. 5). Attempts were also made to quantify the kinetics of the V_j-sensitive transitions between open and residual states, but methodological problems arose. The evaluation of fast transition times (shorter than 1 ms) is limited by frequency characteristics of the amplifier, cell input capacity, pipette-solution capacity, pipette resistance, etc. Thus all of the V_j-sensitive I_j transitions faster than 1 ms were classified as 1-ms transitions. These limitations notwithstanding, a frequency histogram describing the upper limit of the transition time between open and residual states was constructed (Fig. 5 C). On average, the duration of V_j-sensitive I_j transitions was 2 ± 0.2 ms ($n = 105$).

Our findings support the existence in gap junction channels of two distinct gating mechanisms: a fast V_j-sensitive and a slow V_j-insensitive. The I_j transition kinetics observed with CO_2 are similar to those reported for first channel opening during channel formation (Weingart and Bukauskas, 1995; Bukauskas and Weingart, 1994), with V_m gating in insect cells (Bukauskas and Weingart, 1994), and during uncoupling by lipophilic agents (Weingart and Bukauskas, 1995). Consequently, one may postulate that the I_j transitions with slow kinetics are related to a more general phenomenon: chemical gating. We speculate that the "chemical" gating mechanism may be composed of a slow gating element and a complex of sensorial elements. Ca_i^{2+}, H_i^+, or

other cytosolic molecules may act as transducers of various uncoupling factors acting on gap junctions from the cytoplasmic side, whereas lipophilic agents probably act directly on junctional protein (Burt, 1989; Peracchia, 1991; Bastide et al., 1995). In all of these cases the gating effect could be achieved by the activation of the same gating element.

The different kinetics of chemical and V_j gating point to two distinct molecular mechanisms. The slow kinetics of the chemical gate may reflect a more complex conformational change in connexins (Unwin, 1988). Chemical gating may require the participation of 6 or 12 connexins of a connexon or a cell-cell channel, respectively. The gating activity of individual connexins is probably fast, but may not always be synchronous; this may be the cause of the slow kinetics of I_j fluctuation. Recently two gating mechanisms have also been shown by patch clamp in Cx46 hemichannels expressed in oocytes (Trexler et al., 1996). As in gap junctions, these mechanisms had different kinetics, and the slow one closed the hemichannel completely, whereas the other did so only partially.

An interesting question is whether chemical and voltage gates operate independently from each other. Although it is clear that the V_j gate operates independently (Fig. 6 B), as the channels flicker between open and residual states in the absence of chemical treatment, it was not clear whether the chemical gate is independent of the state of the V_j gate. Based on the present study, one may conclude that the chemical gate is able to operate when the voltage gate is either open (Figs. 1 and 6 C) or closed (Figs. 2, 3, and 6 D).

At this stage one can say that although the two gates seem to be able to operate individually, the possibility that the two gating mechanisms influence each other cannot be ruled out.

REFERENCES

Bastide, B., J. C. Hervé, L. Cronier, and J. Délèze. 1995. Rapid onset and calcium independence of the gap junction uncoupling induced by heptanol in cultured heart cells. *Pflügers Arch.* 429:386–393.

Bennett, M. V. L., L. C. Barrio, T. A. Bargiello, D. C. Spray, E. Hertzberg, and J. C. Sáez. 1991. Gap junctions: new tools, new answers, new questions. *Neuron.* 6:305–320.

Bennett, M. V. L., and V. Verselis. 1992. Biophysics of gap junctions. *Semin. Cell Biol.* 3:29–47.

Beyer, E. C. 1993. Gap junctions. *Int. Rev. Cytol.* 137C:1–37.

Beyer, E. C., J. Kistler, D. L. Paul, and D. A. Goodenough. 1989. Antisera directed against connexin43 peptides react with a 43-kDa protein localized to gap junctions in myocardium and other tissues. *J. Cell Biol.* 108:595–605.

Brockes, J. P., K. L. Fields, and M. C. Raff. 1979. Studies on cultured rat Schwann cells. 1. Establishment of purified populations from cultures of peripheral nerve. *Brain Res.* 165:105–118.

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 C. Peracchia. 1996. Uncoupling by CO_2 involves a slow and V_j-insensitive gating mechanism, revealing the existence of two gates in gap junctions. *Mol. Biol. Cell.* 7:280a.

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.

Burt, J. M. 1989. Uncoupling of cardiac cells by doxyl stearic acids: specificity and mechanism of action. *Am. J. Physiol.* 256:C913–C924.

Chow, I., and S. H. Young. 1987. Opening of single gap junction channels during the formation of electrical coupling between embryonic muscle cells. *Dev. Biol.* 122:332–337.

Churchill, D., S. Coodin, R. R. Shivers, and S. Caveney. 1993. Rapid de novo formation of gap junctions between insect hemocytes in vitro: a freeze-fracture, dye-transfer and patch-clamp study. *J. Cell Sci.* 104: 763–772.

Crow, D. S., E. C. Beyer, D. L. Paul, S. S. Kobe, and A. F. Lau. 1990. Phosphorylation of connexin43 gap junction protein in uninfected and Rous sarcoma virus-transformed mammalian fibroblasts. *Mol. Cell. Biol.* 10:1754–1763.

Miyoshi, H., M. B. Boyle, L. B. MacKay, and R. E. Garfield. 1996. Voltage-clamp studies of gap junctions between uterine muscle cells during term and preterm labor. *Biophys. J.* 71:1324–1334.

Moreno, A. P., G. I. Fishman, and D. C. Spray. 1992. Phosphorylation shifts unitary conductance and modifies voltage dependent kinetics of human connexin43 gap junction channels. *Biophys. J.* 62:51–52.

Neyton, J., and A. Trautmann. 1985. Single-channel currents of an intercellular junction. *Nature.* 317:331–335.

Obaid, A. L., S. J. Socolar, and B. Rose. 1983. Cell-to-cell channels with two independently regulated gates in series: analysis of junctional conductance modulation by membrane potential, calcium, and pH. *J. Membr. Biol.* 73:69–89.

Peracchia, C. 1991. Effects of the anesthetics heptanol, halothane and isoflurane on gap junction conductance in crayfish septate axons: a calcium- and hydrogen-independent phenomenon potentiated by caf-
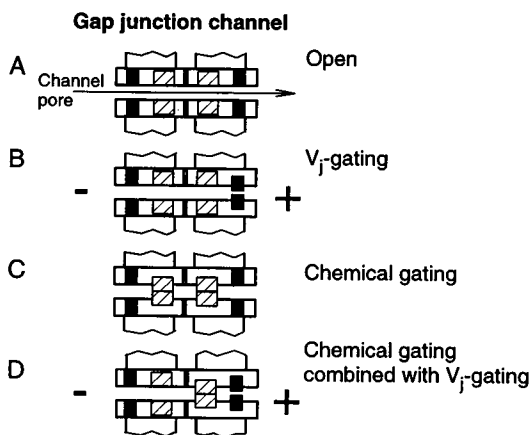
Gap junction channel

FIGURE 6 Schematic model of gap junction channel gates illustrating different functional states, reflecting the state of each gating mechanism. The V_j-sensitive gate, whose operation is characterized by I_j transitions of fast kinetics, is drawn as a black box. The chemical-sensitive gate, the behavior of which is reflected by I_j transitions of slow kinetics, is drawn as a hatched box. Channels in open state, γ_j(main state), have both gates open (A). At the residual state, γ_j(residual state), the V_j gate of one hemichannel is closed (B), but the channel pore remains partially open, because the V_j gate is unable to close the channel completely. In contrast, the chemical gate closes the channel pore completely (C). (D) Effect of CO_2 at high V_j, depicting the structural correlate of slow I_j transitions between residual and closed states. Under these conditions, the chemical gate is believed to completely close a channel that is already partly closed by the V_j gate.

feine and theophylline, and inhibited by 4-aminopyridine. *J. Membr. Biol.* 121:67–78.

Peracchia, C., A. Lazrak, and L. L. Peracchia. 1994. Molecular models of channel interaction and gating in gap junctions. *In* Handbook of Membrane Channels. Molecular and Cellular Physiology. C. Peracchia, editor. Academic Press, San Diego. 361–377.

Spray, D. C., A. L. Harris, and M. V. Bennett. 1981. Equilibrium properties of a voltage-dependent junctional conductance. *J. Gen. Physiol.* 77: 77–93.

Traub, O., R. Eckert, H. Lichtenberg-Fraté, C. Elfgang, B. Bastide, K. H. Scheidtmann, D. F. Hülser, and K. Willecke. 1994. Immunochemical and electrophysiological characterization of murine connexin40 and -43 in mouse tissues and transfected human cells. *Eur. J. Cell Biol.* 64: 101–112.

Trexler, E. B., M. V. L. Bennett, T. A. Bargiello, and R. L. Verselis. 1996. Voltage gating and permeation in gap junction channels. *Proc. Natl. Acad. Sci. USA.* 93:5836–5841.

Unwin, N. 1988. The structure of ion channels in membranes of excitable cells. *Neuron.* 3:665–676.

Verselis, V. K., M. V. L. Bennett, and T. A. Bargiello. 1991. A voltage-dependent gap junction in *Drosophila melanogaster. Biophys. J.* 59: 114–126.

Veenstra, R. D., and P. Brink. 1996. Do connexin channels have a residual conductance state? *Biophys. J.* 70:1082–1084.

Veenstra, R. D., and R. L. DeHaan. 1986. Measurement of single channel currents from cardiac gap junctions. *Science.* 233:972–974.

Veenstra, R. D., H. Z. Wang, E. M. Westphale, and E. C. Beyer. 1992. Multiple connexins confer distinct regulatory and conductance properties of gap junctions in developing heart. *Circ. Res.* 71:1277–1283.

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

Weingart, R., and F. F. Bukauskas. 1995. Regulation of gap junctions by lipophilic agents and ions supports the concept of two gating mechanisms. *Experientia.* 51:A68. (Abstr.)

Willecke, K., H. Hennemann, E. Dahl, S. Jungbluth, and R. Heynkes. 1991. The diversity of connexin genes encoding gap junctional proteins. *Eur. J. Cell Biol.* 56:1–7.