Calmodulin Role in Hemichannel Gating

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Contributor: Camillo Peracchia

Evidence for the existence of connexin hemichannels was first demonstrated in cultured cells expressing connexin43 by data showing that a fluorescent dye enters the cells when the extracellular calcium concentration is reduced. This was proven by evidence that the membrane resistance significantly drops when the cells are bathed in no-added-Ca^{2+} solution. Significantly, while external Ca^{2+} keeps hemichannels closed, an intracellular [Ca^{2+}] rise opens hemichannels. Hemichannel opening is prevented by calmodulin inhibitors, suggesting that calmodulin plays a role in hemichannel gating opposite to that in gap junction channels.

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1. Introduction

The existence of connexin hemichannels was first proven in cultured cells expressing Cx43 by evidence of 5(6)-carboxyfluorescein influx with lowered [Ca^{2+}][1]. Hemichannel permeability proved similar to that of gap junction channels and, similarly, hemichannels were sensitive to octanol and heptanol[1]. We further confirmed the presence of hemichannels by demonstrating that the membrane resistance (Rm) of Novikoff hepatoma cells drastically drops with no-added-Ca^{2+} solutions[1]. In control cells, Rm was lower than in cells transfected with anti-sense Cx43 (~800 and ~4000 MΩ, respectively), proving that the number of open hemichannels in low Ca^{2+} saline is much lower than in controls[1].

2. Role

While external Ca^{2+} clearly plays a major role in keeping hemichannels closed[2][3], an increase in $[Ca^{2+}]_i$ actually causes hemichannel opening[4][5][6]. In Cx32-expressing cells, a $[Ca^{2+}]_i$ rise to ~500 nM, caused by treatment with 2 μM A23187 (a Ca^{2+} ionophore), triggered ATP release and dye uptake that was blocked by a Cx32 mimetic peptide[4]. Significantly, this peptide (“32gap 24”; GHGDPLHLEEVK, res. 110–121) mimics a CL sequence that just precedes the CaM binding site. Hemichannel opening was prevented by W7[4], suggesting a CaM role in hemichannel gating that is opposite its role in cell-to-cell channels. A subsequent study confirmed these data on Cx43 hemichannels expressed in glioma cells and primary glial cells[5]. Surprisingly, however, while a $[Ca^{2+}]_i$ rise to ~500 nM opened hemichannels, this phenomenon vanished with a greater $[Ca^{2+}]_i$ rise. Note, however, that the hemichannel closure at high $[Ca^{2+}]_i$ is likely to be CaM-independent[7]. CaM’s role in hemichannel gating has been also reported for Cx50 hemichannels expressed in HeLa cells[8].

The gating mechanism of hemichannels and the role of CaM in hemichannel opening and closure are still poorly understood. A recent study[6] reported that a CT-deleted Cx32 mutant (Cx32–D220) renders hemichannels less sensitive to $[Ca^{2+}]_i$; significantly, Ca^{2+}-sensitivity is restored by application of the peptide “32gap 24”. These Authors suggested that the interaction of “32gap 24” with the Cx32–D220 hemichannel stabilizes CL fluctuations, and proposed that CL fluctuations may prevent the exposure of CL residues to a target domain relevant to gating[6]. In agreement with our “CaM–Cork” gating model[9][10][11], they believe that Cx32 hemichannels are kept closed at resting $[Ca^{2+}]_i$ by a plugging molecule likely to be a CaM lobe[6]. Also consistent with the idea that a CaM lobe plugs the hemichannels (cork gating), is evidence that the hemichannels are opened by positive (depolarizing) voltage pulses[6][12]. Significantly, Castro and coworkers directly measured the opening and closure of Cx32 hemichannels by patch-clamp in response to a $[Ca^{2+}]_i$ rise, hence the kinetics of the hemichannel’s “cork” unplugging[12]. Indeed, based on the CaM–Cork, model the negatively charged CaM lobe is expected to be displaced out of the positively charged hemichannel’s mouth (vestibule) by membrane depolarization caused by positive voltage pulses[9].

A recent study on a Cx46 mutant (G143R) confirmed the direct CaM role in hemichannel gating[13]. The G143R mutation in the CaM-binding site, which increases hemichannel permeability[14], affected CaM binding to CL2[13]. As predicted, both CaM binding to Cx46’s G143R mutant and increased hemichannel permeability were inhibited by CDZ. Significantly, G143R substitution greatly increases the CaM–Cx46 interaction in the presence and absence of Ca^{2+}[13], confirming that that CaM is anchored to connexins at normal $[Ca^{2+}]_i$[15][16]. Perhaps, the enhanced Ca^{2+}–CaM affinity of the G143R mutant is caused by a slight shift in the site toward the COOH-terminus end[9]. The CaM–Cx46 interaction was also confirmed by immunofluorescent CaM–Cx46 co-localization[13].

Recently, Garcia and coworkers reported that the mutation G12R in Cx26’s NT increases the kinetics speed of slow-gate closure and, although the hemichannels still close completely at a very negative Vm, they are not affected by Ca^{2+}, even though Ca^{2+} still binds[17]. While it might be irrelevant to this phenomenon, it is noteworthy to realize that the G12R mutation reduces the extent of the NT’s CaM-binding site[9]. Another mutation (N14K)[18] decreased the extent of the CaM binding site even more[9]. The N14K mutation raises the energy barrier between open and closed hemichannel states and shifts calcium sensitivity, voltage sensitivity and deactivation time constants[18].

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