Mechanism of the defect in gap-junctional communication by expression of a connexin 26 mutant associated with dominant deafness

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SPECIFIC AIMS

Gap-junctional channels (connexin oligomers) are large-diameter aqueous pores formed by head-to-head association of two gap-junctional hemichannels (connexons), one from each of the adjacent cells. Mutations of connexin 26 (Cx26) are the most frequent cause of genetic deafness. The aim of this work was to elucidate the mechanism of the dominant defect on gap-junctional communication produced by expression of Cx26 R75W. To accomplish this task, we measured gap-junctional channel and hemichannel activities in *Xenopus laevis* oocytes.

PRINCIPAL FINDINGS

1. Cx26 R75W is expressed at the plasma membrane where it forms gap-junctional hemichannels (GJH) that are functional and blocked by extracellular divalent cations

We found that the R75W mutant is expressed at the plasma membrane, but does not form gap junctions permeable to small inorganic ions, which is in agreement with previous reports. However, our results suggested the presence of functional hemichannels that account for cell membrane depolarization in response to lowering medium (Ca^{2+}), and cause cell lysis prevented by increasing extracellular (Ca^{2+}). The presence of functional GJH was confirmed by measurements of whole-cell R75W GJH currents blocked by divalent cations (**Fig. 1*B***). These results show that the absence of gap-junctional communication in cell pairs expressing R75W Cx26 is not due to lack of GJH formation or to lack of connexon stability.

2. The voltage dependence of the WT and R75W Cx26 whole-cell hemichannel currents differs

The voltage dependency of WT and R75W Cx26 GJH currents differed markedly (Fig. 1). The WT Cx26 hemichannel currents did not exhibit significant voltage-dependent inactivation in the voltage range studied (−80 to +40 mV, Fig. 1*A, B*), consistent with the properties of the WT Cx26 GJC. In contrast, the R75W GJH display a rectifying instantaneous I–V relationship, with inactivation at negative voltages and activation at positive voltages (Fig. 1*A, C*). Both WT and R75W Cx26 GJH were blocked by 18βG (Fig. 1*A, B*), as expected from hemichannel data on other connexins.

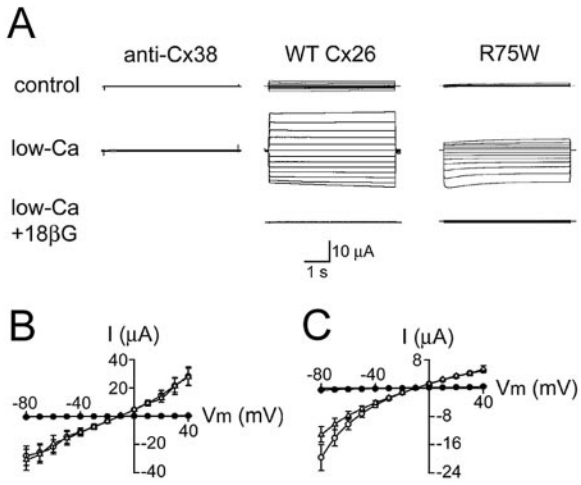

Figure 1. Whole-cell gap-junctional hemichannel activity. *A)* Typical hemichannel currents. Records were obtained in single oocytes bathed with ND96 solution containing 0.7 mM Ca^{2+}/1 mM Mg^{2+} (control) and nominally Ca^{2+}/Mg^{2+}-free solution in the absence (low Ca) or presence of 20 μM 18β-glycyrrhetinic acid (18βG). The whole-cell currents were recorded upon clamping the voltage for 5 s to values between −80 and 40 mV, at 10 mV intervals. *B)* Average WT Cx26 hemichannel current-voltage relationships. Data obtained 0.1 s (○) and 5 s (Δ) after the pulse in the absence (n=9) or presence of 18βG (●, n=8). Data are means ± SE. *C)* Average R75W Cx26 hemichannel current-voltage relationships. See panel *B* for symbols. Data are means ± SE from n = 6.

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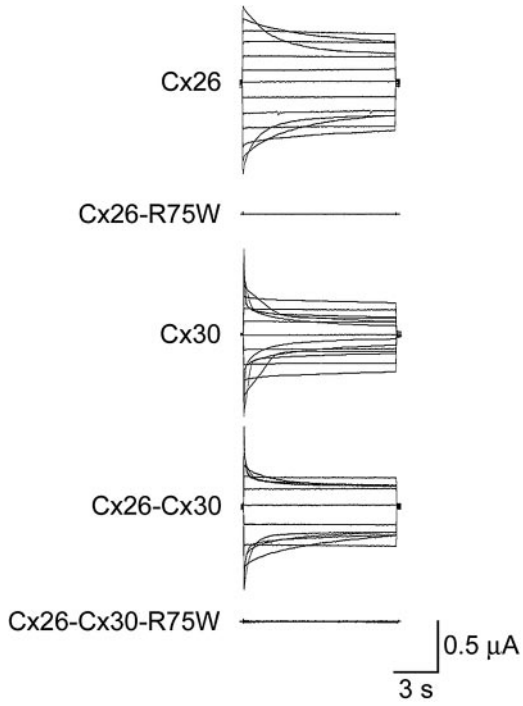

Figure 2. Dominant effect of the R75W mutation. Inhibitory effect of R75W expression on gap-junctional currents measured in oocyte pairs expressing Cx26 or Cx26 and Cx30 (Cx26-Cx30). The oocytes were injected with equal amounts of Cx26, Cx30, and Cx26 R75W cRNA, alone or in the combinations indicated. The cells were clamped at −60 mV, and a transjunctional potential was generated by stepping the voltage of one cell to values between −120 and 120 mV, at 20 mV intervals, with the voltage of the cell clamped at −60 mV was used as reference. The records are typical of 6–12 experiments.

3. Single GJH data indicate that the decreased permeability and altered voltage dependence of R75W GJH in the whole-cell studies are caused by changes in the voltage dependence of the open probability (P_o), with no changes in single-channel conductance (γ)

To identify the biophysical bases of the differences between the whole-cell GJH currents of oocytes expressing WT or R75W Cx26, we carried out studies on excised, inside-out single GJH. The WT GJH displayed a γ of 281 ± 13 pS, consistent with the ~150 pS observed for single gap-junctional channels (two Cx26 GJH in series). The records showed open-channel noise similar to that of Cx26 gap-junctional channels (GJC) and several subconductive states between the fully open and closed states. These properties of the WT Cx26 single-GJH are consistent with the data available for single gap-junctional channels. The γ of the fully open R75W single GJH (300±8 pS) was not different from that of the WT Cx26 single GJH (281±13 pS, see above) and was almost linear in the range of ± 40 mV. However, the dwell time of R75W GJH in the fully open state was significantly shorter at +40 mV than at −40 mV. Therefore, the single GJH data indicate that the decreased permeability and altered voltage dependence of R75W GJH in the whole-cell studies are caused by changes in the voltage dependence of P_o, with no changes in γ.

4. Coinjections of WT Cx26, WT Cx30 and R75W Cx26 cRNA into the oocytes indicate that the effect of R75W expression is dominant at the GJC, but not at the GJH level

Figure 2 shows that coexpression of Cx26 R75W inhibits the gap-junctional communication mediated by WT Cx26 or heteromeric WT Cx26-WT Cx30 GJH. These results extend previous observations and are consistent with the dominant nature of the deafness associated with Cx26 R75W. In oocytes injected with equal amounts of WT Cx26 and R75W cRNAs, the hemichannel currents were similar to those of WT Cx26 GJH. These oocytes, however, cannot form GJC (Fig. 2), and therefore the R75W phenotype is dominant at the GJC, but not at the GJH level. These results suggest that the R75W mutation is associated with a defect in the docking of functional hemichannels.

CONCLUSIONS AND SIGNIFICANCE

We conclude that the absence of gap-junctional communication between the cells that form part of the extensive gap-junctional network of the cochlea by expression of R75W Cx26 mutant is fundamentally a consequence of the presence of GJH that cannot form functional GJC. The dominant nature of the R75W mutation is evident only by the absence of functional GJC, and not at the level of formation of functional GJH (**Fig. 3**). It is possible that the loss of effective gap-junctional communication causes cell damage and deafness as a result of a loss of GJC function, causing alterations in K^+ recycling or cell-to-cell transport of metabolites. Since the R75W mutant is well expressed

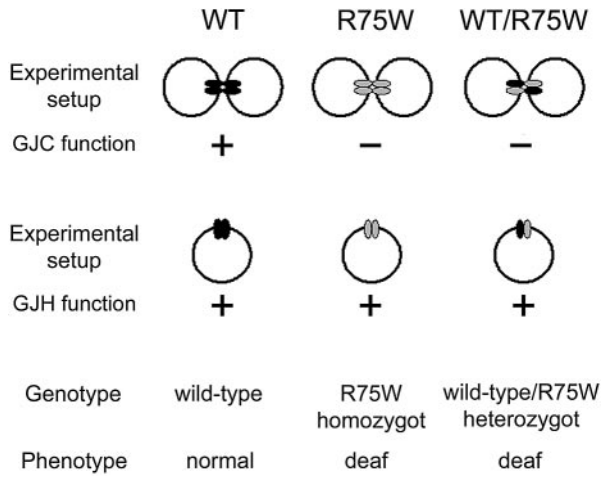

Figure 3. Schematic diagram summarizing of the results. The circles represent individual oocytes in the paired-cell or single-oocyte configurations.

at the plasma membrane and previous observations suggest that R75W can form anatomic plaque-like structures, a role of uncoupled R75W GJH in the death of cochlear cells is also possible. In this case, cell damage and deafness will be a consequence of a gain of GJH function, resulting in a leak communicating the cytoplasm with the extracellular medium. The presence of permeable GJH is associated with cell lysis, and there is accumulating evidence for a role of Cx43 GJH in the cell damage that occurs in conditions such as hypoxia in a variety of cells, including epithelial, muscle and neural cells.

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