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RESEARCH NEWS

Deafness-associated mutation opens the gate to understanding

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New JGP study uncovers a connection between Connexin 26 fast and slow gates.

Gap junctions connect neighboring cells to allow the exchange of ions and metabolites. They are formed by connexins, proteins that assemble into hexameric structures called "hemichannels" in the plasma membrane. A hemichannel in one cell paired with another in a neighboring cell forms a gap junction, but lone hemichannels also function to allow material exchange with the extracellular space. Importantly, impairment of connexins often results in disease. For example, mutations in Connexin 26 (Cx26) are associated with deafness and with syndromes in which deafness is accompanied by problems in skin and other tissues. In this issue of the *Journal of General Physiology*, García et al. explore how a syndromic deafness-associated mutation affects Cx26 activity, and they provide new information about how the channel's activity is regulated (1).

Hemichannel opening is regulated to prevent uncontrolled leakage of ATP and other materials through the pore; the channels only pass current within a narrow voltage range. Because of the closing of the voltage-sensitive "slow gate," Cx26 hemichannels cannot pass current at the negative membrane potentials that most cells experience while at rest. The structural changes underlying slow gate closure are unknown, but research suggests that closure is aided by binding of calcium ions to the extracellular surface of the channel (2). Additionally, currents are reduced at very positive membrane potentials because these voltages cause the cytoplasmic N terminus to move into and partially block the pore (3). It's unclear whether pore restriction by this "fast gate" is related to the slow gate mechanism.

Several mutations associated with deafness are located in the N-terminal portion of Cx26 (4, 5, 6). N-terminal mutations that cause syndromic deafness permit Cx26 to coassemble with connexin 43, creating hyperactive hemichannels that may drive disease (4). But mutations can also change the activity of Cx26 hemichannels in the absence of Connexin 43. For example, hemichannels assembled from Cx26 bearing the syndromic deafness mutation G12R, which substitutes an arginine for the glycine at residue 12, show increased activity compared with WT (4, 5).

Isaac E. García (left), Osvaldo Alvarez (middle), Ramon Latorre (right), et al. explore how the Cx26 syndromic deafness mutation G12R affects hemichannel activity (see graph) and gating. Photos courtesy of the authors.

"I was very interested in the mutation G12R, because it can promote the syndromic phenotype of the disease, but if you put valine in this position, you get nonsyndromic deafness," explains Isaac E. García, an associate professor at the Universidad de Valparaíso, Chile. While a postdoc in Ramon Latorre's laboratory at Valparaíso, García explored how G12R affects Cx26 behavior.

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Consistent with earlier studies, García and colleagues found that G12R mutant hemichannels expressed in frog oocytes passed more current than WT Cx26 hemichannels. Whereas WT Cx26 current dropped at very positive voltages, G12R hemichannel current continued to climb, indicating that the fast gate was defective. Molecular dynamics simulations suggested that the mutation may displace the N terminus toward the cytoplasm and simultaneously promote an interaction with another arginine at residue 99 that prevents the N terminus from moving into and narrowing the pore. Accordingly, changing R99 to an alanine restored G12R hemichannels' fast gate function.

"But we also found that the slow gating mechanism is affected. We found the kinetics of slow gate closure in the mutant are faster than those in WT. This was an unexpected result," says García. G12R hemichannels could still close fully at very negative membrane potentials, but although calcium bound as normal to G12R hemichannels, the channels were insensitive to calcium. Neutralizing the G12R mutation by swapping in alanine at R99 also restored slow gate function.

These data, say García et al., suggest that operation of the fast and slow gates may both involve the protein's N terminus. The two mechanisms may even be allosterically coupled. García and colleagues are already planning to study other deafness mutations and other regions of the protein for more insights into connexin gating.

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