Gating of gap junction channels as revealed in cells stably transfected with wild type and mutant connexin cDNAs

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INTRODUCTION

Gap junction channels are formed of multiple copies of proteins termed connexins. cDNAs encoding three mammalian gap junction proteins (named connexins 26, 32, and 43, according to predicted molecular weights of encoded proteins) have recently been cloned and sequenced (see 1 for review). Although numerous studies have described junctional properties between cells in which one or another connexin type predominates (see 2, 3 for overviews), many connexins display patterns of overlapping tissue expression (e.g., 4). To identify the physiological properties of gap junction channels formed of specific connexins and expressed in the same cell type, we have voltage clamped pairs of communication-deficient cells (SKHep1, a highly metastatic human hepatoma line) stably transfected with wild type or mutant connexin cDNAs along with the selectable marker gene, *neo* (5, 6). In this paper we compare the properties of gap junction channels formed of rat connexin32 and wild type and mutant human connexin43.

RESULTS AND DISCUSSION

We have obtained transfectants using vectors containing either SV40 or RSV promoters (5, 6); antibiotic (G418) resistant colonies were injected with Lucifer Yellow, and well-coupled colonies were picked for subsequent experiments. Connexin expression has been stable for as long as 30 passages, as evidenced by electrophysiological recordings and immunocytochemistry.

The parental cell line exhibited a very low level of endogenous gap junction channel expression. In one series of 86 cell pairs, only 15% showed any channel activity whatsoever, and junctional conductance (g_j) was in no case higher than 0.6 nS (3). Unitary conductance of these infrequent events was ~30 pS (Fig. 1, *top*). In another series of experiments on a clone of SKHep1 cells showing more frequent expression of a small number of endogenous channels, the endogenous channel was found to be voltage dependent, with V_0, the voltage at which g_j is reduced by half, ~20 mV (7). The parental cell line expresses no detectable connexin 26, 32, or 43, and we believe that the endogenous channels represent low level expression of an as yet unidentified member of the connexin family.

Junctional conductance in the transfected cells averaged ~10 nS for rat connexin32 and ~6 nS for human connexin43. To measure unitary junctional conductance (γ_j), g_j was reversibly reduced by exposure to halothane, which does not itself change γ_j (3, 8). Single-channel conductances in cell pairs transfected with connexins 32 and 43 were strikingly different from one another and from the occasional endogenous channel (Fig. 1 *middle, bottom*). For connexin32, γ_j values were 120–150 pS; for connexin43, two channel sizes were resolved, 60 and 90 pS, with the smaller channels being favored after phosphorylating treatments (9). These sizes are comparable to values obtained in systems where these connexins are endogenously expressed (2, 3). Preliminary experiments on connexin26 expressed in another communication-deficient cell type (pc12 cells) suggest that the unitary conductance is ~70 pS (10).

Voltage dependence of connexins 32 and 43 expressed in the same mammalian cell line differed strikingly (Fig. 2), and was also substantially different from results obtained in *Xenopus* oocyte expression studies (e.g., 11). For connexin32 transfectants, g_j was maximal at 0 mV transjunctional voltage (V_j) and declined exponentially with long V_j pulses of either polarity; equilibrium g_j values decreased symmetrically about the 0 mV axis, with V_0 (the voltage at which g_j was reduced by 50%) ~±30 mV, g_{min} (the fraction of g_j that is voltage-insensitive) ~10%, and n (the number of equivalent gating charges) ~2. For connexin43, V_0 is ~ ±60 mV, g_{min} is ~40–50%, and n is also ~2. Preliminary experiments indicate that voltage dependence of connexin26 is also substantial. These data on connexins with markedly different carboxyl terminal regions suggest that the

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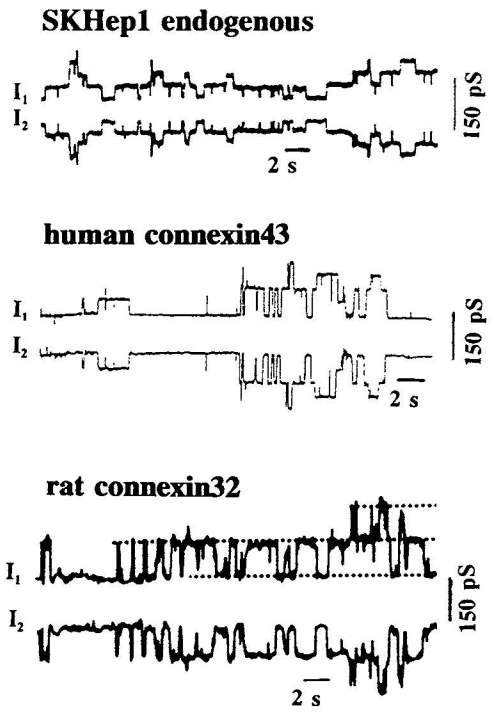

FIGURE 1 Unitary conductances recorded between cells from the parental cell line (SKHepl endogenous) and clones stably transfected with human connexin43 and rat connexin32. Note that traces are scaled so that calibrations are similar, although driving forces for the different traces were 36, 30, and 46 mV, respectively. Using internal solutions in which CsCl was the major salt, unitary conductance of endogenous channels averaged ~30 pS, connexin43 channels ~60 and 90 pS, and connexin32 channels ~130 pS.

length of the carboxyl-terminus is not the major determinant of gating of g_j by voltage.

To determine whether differences in γ_j or voltage dependence might be ascribable to the carboxyl terminal portion of the connexin molecules, we transfected SKHepl cells with mutant connexin43 cDNAs in which a stop codon was inserted so as to encode connexin proteins similar in length (but not in sequence) to connexins 26 and 32 (12). Single-channel studies with the connexin26 analogue obtained γ_j values ~50 pS, whereas the connexin32 analogue showed channels ~150 pS in size. These γ_j values were similar to those of their homologues, suggesting that the carboxyl terminal portion of connexin43 may provide an important determinant of γ_j. Voltage dependence of the truncation mutants, however, was similar to that of the wild type connexin43 (12).

The third transmembrane domain of the connexins is the most amphipathic and thus most likely to line the pore; site-directed mutagenesis of SER158 (which is conserved among the connexins) to PHE, LYS, and ASP

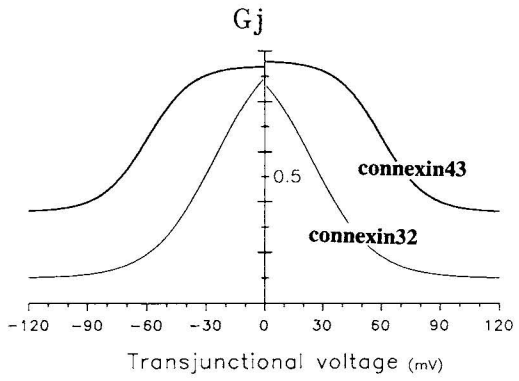

FIGURE 2 Voltage dependence of normalized junctional conductance (G_j) in stable transfectants expressing connexin43 or connexin32. Each curve represents a Boltzmann fit to means of 10 separate preparations. Voltage dependence parameters are given in the text.

led to electrically well-coupled transfectants with markedly reduced permeability to intracellularly injected Lucifer Yellow. In the LYS substitution, γ_j values were higher than for the wild type connexin43 channels. Thus, assignment of M3 as the channel-lining domain is supported; selectivity studies underway should resolve the apparent paradox between increased γ_j and reduced dye permeability.

Together, these studies indicate that the biophysical properties of connexin channels are distinct from one another (and in mammalian cells their properties also differ from those of the same connexins expressed in *Xenopus* oocytes). Continued examination of the same parental cell line stably transfected with these and other connexin constructs should reveal domains responsible for gating mechanisms and provide insight into mechanisms of ion permeation through gap junction channels.

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