ABSTRACT

Cyclic nucleotide-gated (CNG) channels in retinal photoreceptors play a key role in vertebrate phototransduction. The ligand sensitivity of photoreceptor CNG channels is adjusted during and in response to parasite signals, but the mechanisms involved in this process are only partially understood. Heteromeric A3/B3 (α-cone) and A1/B1 (β-cone) channels are sensitive to regulation by phosphoinositides (PIP3 or PIP2), demonstrating a decrease in apparent affinity for cGMP. To determine what subtypes are necessary for PIP3 sensitivity, we generated heteromeric channels by co-expression of PIP3-insensitive A2N (Brady et al., 2006) with B3 or B1 subunits. Using patch-clamp techniques in inside-out configuration, we found that both channel types were insensitive to PIP3, indicating that A3 or A1 but not B3 or B1 subunits confer phosphoinositide sensitivity to heteromeric channels. Consistent with this, co-expression of A3 with B1 or A1 with B3 formed channels that were sensitive to PIP3 regulation. Unlike heteromeric A1 or A2 channels, A3-only channels paradoxically did not show a decrease in apparent affinity for cGMP after PIP3. However, PIP3 induced a nearly three-fold increase in cAMP efficacy for A3 channels, an effect that was reversed by poly-lysine application. The PIP3-dependent change in cAMP efficacy was ablated by a mutation of a critical ligand-displacement residue (DE698) or by truncation of the channel distal to the cyclic nucleotide-binding domain (Δ80 A3). Furthermore, the apparent-cGMP affinity of A3+Δ80 A3 channels was reduced three-fold by PIP3, this change in cGMP sensitivity also was reversed by poly-lysine. Together, these results suggest that regulation of A3 subunits by PIP3, exhibits two components, one of which is regulated either by assembly with B3 subunits or by deletion of the C-terminal region of A3.

RESULTS

FIGURE 1: Structural elements necessary for phosphoinositide sensitivity of photoreceptor CNG channels appear to reside in CNGA3 and CNGA1 (not CNGB3 and CNGB1) subunits.

(a) Representative current traces elicited by 100 mM cGMP (green), or 10 mM cGMP (red) before and after PIP3, for CNGA3-D609M channels. (B) Effects of PIP3 application on PIP3-dependent change in cAMP efficacy for CNGA3 channels with mutation of critical ligand-displacement residue (DE698). Data are means ± SEM of 5-6 independent experiments. *P < 0.05 compared to wild-type A3 channels.

FIGURE 2: PIP3 application increased the relative cAMP efficacy for CNGA3 homomeric channels.

(a) Representative current traces for CNGA3 homomeric channels, elicited by saturating concentrations of cGMP (1 mM green) or cAMP (10 mM red), before and after PIP3 application, and after subsequent poly-lysine (poly-K) application. Currents were elicited by voltage steps from a holding potential of −60 to +60 mV, trace to −60 mV and −30 mV. Leak currents in the absence of cyclic nucleotide were subtracted. (B) Time course for change in cAMP efficacy following PIP3 application induced by 1 μM PIP3, reversed by poly-lysine. Horizontal bars indicate times of application of respective agents.

FIGURE 3: Mutation of critical ligand-selectivity residue (DE698) in cyclic nucleotide-binding domain of CNGA3 alters ligand-specific sensitivity to PIP3.

(a) Representative current traces elicited by 100 mM (red) and 10 mM (green) cGMP before and after PIP3 for CNGA3-D609M channels. (B) Effects of PIP3 application on cGMP sensitivity of photoreceptor CNG channels by using data from strand-crossing experiments. PIP3-sensitive (Δ80 A3) and PIP3-insensitive (A3Δ80) channel currents were normalized to levels in untreated cells expressing wild-type channels. Data are means ± SEM of 4 independent experiments. *P < 0.05 compared to wild-type A3 channel.

REFERENCES


