

Abstract

Hyperpolarization-activated and cyclic nucleotide-gated (HCN) channels were studied in the rat retina. The expression pattern of the four HCN channel isoforms HCN1 – 4 was studied using immunocytochemistry with isoform-specific antibodies and confocal microscopy. Using the patch-clamp technique, functional properties of HCN currents were recorded in 240 voltage-clamped bipolar cells and 20 photoreceptor cells in a retinal slice preparation. While recording, cells were filled with Lucifer Yellow. On the basis of morphological and electrophysiological criteria, recorded and filled bipolar cells could be classified as 11 types of cone bipolar cells and one type of rod bipolar cell. Two of the cone bipolar cell types had not been identified in previous studies.

Based on immunohistochemical and electrophysiological results, HCN1 was found in rod and cone photoreceptors. Cones also expressed HCN3 at the cone pedicle base. Type 3 bipolar cells expressed HCN4, type 5 and type 6a bipolar cells HCN1 and HCN4. These isoforms were found throughout the bipolar cells. Strikingly, HCN2 was highly concentrated at the axon terminal systems of type 3, 5, 6, 7, 8, and 9 cone bipolar cells and the rod bipolar cell. No HCN immunoreactivity was found in type 1, 2, and 4 cone bipolar cells. In the electrophysiological recordings, the activation kinetics and the mid-point potential of activation were studied in detail. In general, the properties of HCN currents recorded from the different bipolar cell types in the retinal slice agreed reasonably well with those of the respective isoforms expressed heterologously in HEK293 cells. All HCN currents could be blocked by application of external cesium. Cones and cone bipolar cells type 5 and 6a expressed more than one HCN isoform, however, electrophysiologically, no evidence for the formation of heterooligomeric channels was found. In some instances, functional properties of HCN channels in retinal cells differed from those of the heterologously expressed channel isoforms, indicating that the channels might be modulated in the retina. Preliminary experiments suggest that intracellular calcium and cAMP might be involved in the modulation of the channels.

The widespread expression of HCN channels in the retina suggests that they are involved in retinal information processing at several stages. The most striking feature of HCN channel distribution was the synaptic localization of HCN2 and HCN3. This might indicate that the channels are involved in regulation of synaptic transmission.