Abstract

Olfactory signal transduction takes place in the cilia of olfactory neurons. Ca^{2+}-activated Cl^--channels participate in the generation of action potentials in these cells. The channels conduct a depolarizing chloride current. Cloning of olfactory Ca^{2+}-activated Cl^--channels has not been successful, yet. To identify these channels molecularly we used the ODORA cell-line which is of olfactory origin. ODORA cells express olfactory Ca^{2+}-activated Cl^--channels and several other olfactory "marker" proteins. It is assumed that Bestrophin proteins are likely candidates to form functional Ca^{2+}-activated Cl^--channels. Therefore I cloned members of the Bestrophin gene-family from ODORA cells.

I successfully isolated four full-length Bestrophin cDNAs. The cDNAs were transiently transfected into HEK 293 cells. The proteins were detected by hemagglutinin (HA)-Tag immunostaining. Stably transfected cell-lines were established that express each Bestrophin isoform constitutively. Electrophysiological recordings showed that Bestrophin 1 forms Ca^{2+}-activated Cl^--channels. Isoform-specific antibodies were raised against all four Bestrophins. In Western Blots, Bestrophin proteins were detected by α-HA-antibodies as well as by α-Bestrophin-antibodies in membrane fractions isolated from stably transfected cell lines.

The Ca^{2+}-activated Cl^--channel in ODORA cells most likely is regulated by CaM. Therefore Bestrophins were examined for potential CaM interaction. All Bestrophin proteins bind to CaM-Agarose and could be eluted with Ca^{2+}-free buffer. Amino-acid substitutions in a 1-5-8-14 CaM binding-site of Bestrophin 2 and 3 did not change the interaction to the CaM matrix. Immunostainings of tissue sections showed that Bestrophin 2 is expressed in olfactory neurons, preferentially in the dendrites and the soma. In some preparations, olfactory cilia were also stained. Because Bestrophin 2 is only moderately co-localized with cyclic nucleotid-gated (CNGA2) channels, it is still uncertain whether Bestrophins form Ca^{2+}-activated Cl^--channels in the olfactory epithelium.