

Minireview

Cyclic nucleotide-gated ion channels in sensory transduction

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Abstract Cyclic nucleotide-gated (CNG) channels, directly activated by the binding of cyclic nucleotides, were first discovered in retinal rods, cones and olfactory sensory neurons. In the visual and olfactory systems, CNG channels mediate sensory transduction by conducting cationic currents carried primarily by sodium and calcium ions. In olfactory transduction, calcium in combination with calmodulin exerts a negative feedback on CNG channels that is the main molecular mechanism responsible for fast adaptation in olfactory sensory neurons. Six mammalian CNG channel genes are known and some human visual disorders are caused by mutations in retinal rod or cone CNG genes. © 2006 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

Keywords: Transduction; Sensory; Adaptation; Olfaction; cAMP; Channelopathy

1. Introduction

Ion channels directly gated by cGMP in retinal rods were first discovered 21 years ago [1]. Fesenko and collaborators [1] showed that cGMP directly activates a current in isolated patches of outer segment membrane of retinal rods. The current was activated by cGMP alone, without the presence of other factors such as ATP or kinases. Moreover, the cGMP-gated current could not be activated by AMP, GMP, ATP or GTP. The cGMP-gated current was shown to be cationic, and the dependence of current activation from cGMP concentration was well described by a Hill function with a coefficient of 1.8 [1]. The discovery of ion channels directly gated by cGMP occurred while studying the molecular mechanisms of phototransduction. Both Ca^{2+} and cGMP were considered as possible second messengers mediating the electrical response to light. However, the action of cGMP was at first believed to be indirect, because in those years the dogma that cyclic nucleotides acted on proteins only by phosphorylation mediated by a cyclic nucleotide-dependent kinase was still standing. Fesenko et al. [1] contributed to unravelling the molecular mechanisms of phototransduction showing that cGMP can directly activate ion channels. Similar channels were also found

in cone photoreceptors [2]. Indeed in photoreceptors cGMP keeps ion channels open in the dark, allowing a continuous influx of Na^+ and Ca^{2+} (dark current), and Ca^{2+} is in turn extruded by a $\text{Na}^+-\text{Ca}^{2+}-\text{K}^+$ exchanger. Light absorption by rhodopsin triggers an enzymatic cascade that leads to the activation of a phosphodiesterase that hydrolyses cGMP to GMP and therefore produces a decrease in the cGMP-gated current. As a consequence photoreceptors hyperpolarize in response to light with a subsequent decrease in glutamate synaptic release (reviewed in [3,4]).

In 1987, Nakamura and Gold [5] detected a similar ionic current directly activated by cGMP or cAMP in the cilia of olfactory sensory neurons. Odorant molecules bind to odorant receptors and activate an enzymatic cascade that leads to an increase in the concentration of cAMP in the cilia, as will be described later in more detail (Fig. 1). Cyclic nucleotide-gated (CNG) channels have also been described in other neuronal and non-neuronal cells (reviewed in [6]).

2. CNG channel subunits

Kaupp et al. [7] first cloned the gene encoding for the CNG channel in bovine retinal rods beginning investigations at the molecular level of the physiological and biophysical properties of these ion channels. At present, six CNG channel genes have been identified in mammalian genomes. These genes code for four types of “A” subunits and two types of “B” subunits [8]. CNG channels are composed of four subunits forming a tetramer with a central pore. The topology of each subunit is similar to that of the cationic voltage-activated channels with six transmembrane spanning domains, a pore-loop domain between the fifth and sixth transmembrane domain, and intracellular N- and C-terminal regions. CNG channels are activated by the direct binding of cyclic nucleotides to a large C-terminal cyclic nucleotide-binding domain and are only weakly sensitive to membrane voltage (Fig. 3E).

Unfortunately, different nomenclatures for the CNG subunits have been used for several years in different laboratories, and therefore the reading of most of the previous papers may result confusing. We will use the most recent commonly agreed nomenclature for CNG channels [8] and indicate previous names in parenthesis.

Native retinal rod channels are composed of two types of subunits: CNGA1 ($\alpha 1$, CNG1, RCNC1) and CNGB1 ($\beta 1$, CNG4, RCNC2) [7,9] with a stoichiometry of three CNGA1 and one CNGB1a (a B1 splice variant) subunits [10,11]. Retinal cone channels are also composed of two types of subunits: CNGA3

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Abbreviation: CNG, cyclic nucleotide-gated

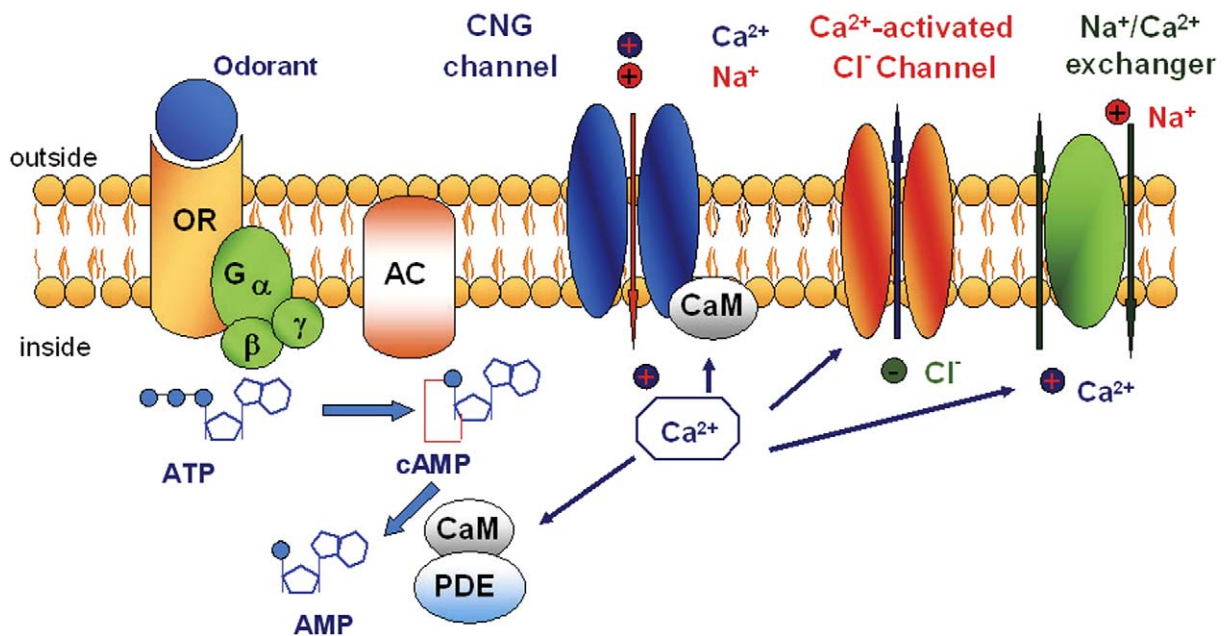


Fig. 1. Olfactory transduction in the cilia of olfactory sensory neurons. Odorant molecules bind to odorant receptors (OR) in the ciliary membrane activating a G-protein (G) that, in turn, stimulates an adenylate cyclase (AC). cAMP directly gates CNG channels causing an odorant-induced inward current carried by Na^+ and Ca^{2+} ions. The increased Ca^{2+} concentration in the cilia causes the opening of Ca^{2+} -activated Cl^- channels and the subsequent Cl^- efflux which further depolarizes the cell. Intracellular Ca^{2+} also binds to calmodulin (CaM) lowering the ligand sensitivity of the cAMP-gated channels. Ca^{2+} -calmodulin also stimulates the activity of a phosphodiesterase (PDE). Ca^{2+} is extruded by a $\text{Na}^+/\text{Ca}^{2+}$ exchanger.

($\alpha 2$, CNG3, CCNC1) and CNGB3 ($\beta 2$, CNG6, CCNC2) [12,13] with a stoichiometry of two CNGA3 and two CNGB3 subunits [14]. Native channels of olfactory sensory neurons are instead composed of three types of subunits: CNGA2 ($\alpha 3$, CNG2, OCNC1), CNGA4 ($\alpha 4$, CNG5, OCNC2, CNGB2) and CNGB1 ($\beta 1$, CNG4, RCNC2) [15–19] with a stoichiometry of two CNGA2, one CNGA4 and one CNGB1b (a B1 splice variant) [20] (Fig. 3E). The subunits CNGA1, CNGA2, and CNGA3 when expressed alone in heterologous systems can form a functional channel activated by cyclic nucleotides, whereas the other subunits cannot form functional channels but have a physiologically relevant modulatory role. Indeed, electrophysiological studies measuring the functional properties of the heterologously expressed principal subunits showed several differences with the respective native channels such as activation by cyclic nucleotides, ion permeation, sensitivity to blockers, and regulation by the complex Ca^{2+} -calmodulin, suggesting that additional subunits and/or modulatory components were still to be discovered [6,21,22]. Cloning of the genes of additional subunits allowed the study of the influence of the subunit composition on the channel properties [6,23–25]. As an example, Fig. 3C shows the comparison between currents activated by cAMP in homomeric CNGA2 channels expressed in HEK293 cells and native olfactory channels. The concentration of cAMP necessary to obtain 50% of the maximal current activation of homomeric CNGA2 channels was about 30 μM , while in heteromeric native channels was about 3 μM , corresponding to a 10-fold higher sensitivity for cAMP of native channels compared with homomeric CNGA2 channels.

The CNG subunit composition of retinal rods, cones or olfactory sensory neurons is tuned to a specific physiological role in sensory transduction. In this review, we will primarily focus on the olfactory CNG channel.

3. Olfactory transduction and adaptation

In vertebrates, volatile odorant molecules reach the olfactory epithelium in the nasal cavity and interact with odorant receptors located in the cilia of olfactory sensory neurons, where olfactory transduction occurs. Olfactory sensory neurons are bipolar neurons with a single dendrite that reaches the surface of the epithelium and terminates with a knob from which several cilia protrude. The binding of odorant molecules to odorant receptors [26] in the cilia triggers an enzymatic cascade that leads to an increase in the intraciliary concentration of cAMP (Fig. 1). cAMP causes the opening of the ciliary CNG channels that allow an influx of Na^+ and Ca^{2+} inside the cilia. Ca^{2+} -activated Cl^- channels are then activated in turn and, due to the unusually high intracellular Cl^- concentration, produce the outflow of Cl^- , contributing to the inward current [27]. As a result of the odorant binding the olfactory sensory neuron depolarizes. The depolarization spreads passively to the dendrite and soma of the neuron, triggering action potentials that are conducted along the axon to the olfactory bulb [28–30]. The olfactory CNG channel allows Ca^{2+} entry not only for excitatory but also for inhibitory effects [31]. The complex Ca^{2+} -calmodulin activates a phosphodiesterase (PDE1C2) that hydrolyzes cAMP [32,33], and also produces a negative feedback effect on the CNG channel itself, that has been shown to mediate olfactory adaptation [34]. Indeed, during short repetitive exposures to odors, olfactory sensory neurons rapidly adapt to the stimulus by decreasing their responsiveness in a Ca^{2+} -dependent manner [35]. Kurahashi and Menini [36] investigated the localization of the principal molecular mechanism for adaptation in the olfactory transduction process. To determine whether the response reduction in the adapted state was attributable to a reduction in the cAMP

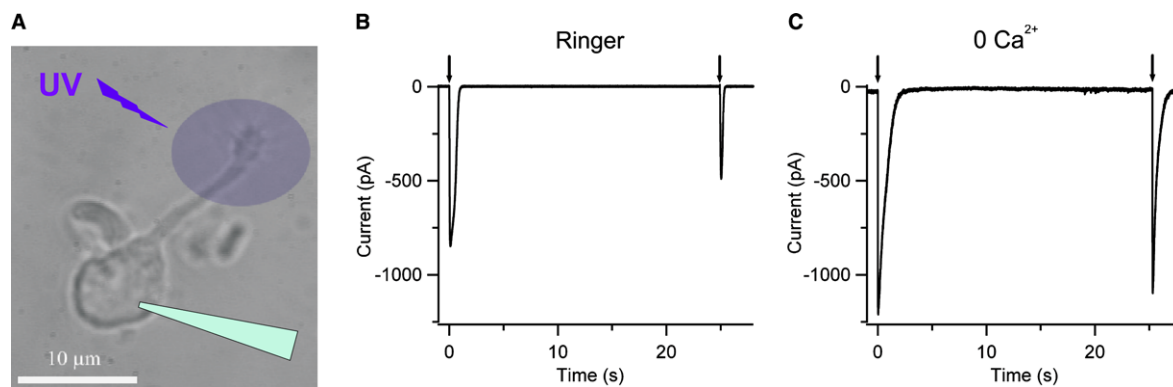


Fig. 2. Odorant adaptation in an isolated mouse olfactory sensory neuron. (A) Patch-clamp in the voltage-clamp whole-cell configuration was used to record currents from isolated mouse olfactory sensory neurons. Caged cAMP was included in the intracellular solution filling the patch pipette. Within a few minutes after rupture of the patch to establish the whole-cell configuration, the caged cAMP diffused into the cilia. Ultraviolet light flashes were applied to release the physiologically active cAMP into the ciliary region. (B) and (C) Responses at -50 mV to two identical ultraviolet light flashes releasing the same concentration of cAMP were recorded in the same neuron in a Ringer or in a nominally 0 Ca^{2+} solution (10 mM EGTA) in the same neuron. In Ringer (B), adaptation was observed, as the peak amplitude of the response to the second flash (-489 pA) was about 58% of the response to the first flash (-842 pA). In a nominally 0 Ca^{2+} solution (C) adaptation was abolished (unpublished results from Boccaccio et al. [38]).

production or was instead due to other processes occurring after the production of cAMP, CNG channels in intact neurons were directly activated by flash photolysis of caged cAMP (Fig. 2). The ciliary cytoplasm was loaded with caged cAMP through diffusion from a patch pipette. Application of ultraviolet light flashes to the ciliary region caused the photolysis of caged cAMP, thereby producing rapid and repeatable increments in cAMP concentration. Therefore, cAMP-gated channels could be directly activated, bypassing the early stages of odorant transduction (i.e. odorant receptor activation and G-protein and adenylate cyclase signalling, Fig. 1). Fig. 2 shows the results of an experiment illustrating that adaptation was measured with repetitive caged cAMP photolysis and that extracellular Ca^{2+} was necessary for adaptation. In physiological Ringer solution the peak of the current response to the second flash was reduced to about 58% of the control response (Fig. 2B), whereas in a nominally 0 Ca^{2+} solution the current responses to repetitive flashes were almost identical. Kurahashi and Menini [36] showed that cAMP- and odorant-induced responses had similar adaptation properties, indicating that the entire adaptation process takes place after the production of cAMP and might be mediated by Ca^{2+} -calmodulin-dependent inhibition of the olfactory CNG channel. Experiments on olfactory adaptation performed in knock-out mice of the CNGA4 subunit confirmed that the molecular mechanism for adaptation is localized at the channel level, most likely through CNG channel inhibition by Ca^{2+} -calmodulin [37].

Recent experiments were also performed to investigate if the Ca^{2+} -calmodulin-induced activity of the PDE (Fig. 1) is required for olfactory adaptation. Using the poorly hydrolysable caged 8-Br-cAMP and the PDE inhibitor IBMX it has been shown that an increase in PDE activity is not necessary for adaptation [38], further supporting the notion that the Ca^{2+} -mediated negative feedback on the olfactory CNG channel is the main molecular mechanism responsible for fast adaptation. At present the overall molecular picture of how Ca^{2+} causes adaptation is the following: Ca^{2+} enters the cilia through the CNG channels and the channel sensitivity to cAMP is significantly reduced by Ca^{2+} -calmodulin modulation. As a consequence, CNG channel open probability could

be significantly reduced. Thus, in the adapted state, the same cyclic nucleotide concentration as in the control state produces a lower CNG channel open probability and therefore a smaller current (Fig. 2). A recent mathematical model of adaptation based on direct negative regulation of CNG channels by Ca^{2+} -calmodulin in olfactory cilia well reproduces the experimental data [39].

4. Modulation of CNG channels by Ca^{2+} -calmodulin

Studies of native CNG channels have shown that the addition of micromolar concentrations of intracellular Ca^{2+} was able to decrease the channel sensitivity to cGMP or cAMP, probably by activating a Ca^{2+} -responsive endogenous factor already pre-associated with the channel [40–46]. Bradley et al. [44] have recently shown that Ca^{2+} -free calmodulin, called apocalmodulin, is able to bind to the heterologously expressed heteromeric olfactory CNG channels even in the absence of Ca^{2+} . Moreover, when Ca^{2+} concentration rises above 100 nM, Ca^{2+} can rapidly modulate the CNG channel sensitivity by directly binding to the pre-associated calmodulin. Furthermore, it was suggested [44] that also in native channels the pre-associated endogenous factor could be apocalmodulin, although a demonstration is still missing. Since Ca^{2+} enters into the olfactory cilia through the CNG channel itself, the pre-associated Ca^{2+} -responsive factor could provide a very fast feedback modulation at the channel level.

Early works [47–51] have identified in the N-terminus of CNGA2 a classic basic amphiphilic α -helix (Baa) motif with high affinity for Ca^{2+} -calmodulin and have shown that the sensitivity to cAMP of heterologously expressed homomeric CNGA2 channels was decreased by the binding of Ca^{2+} -calmodulin to the Baa motif. However, in recent years, there has been a considerable progress in elucidating the molecular events producing modulation of the native channels and it has been shown that the Baa motif of CNGA2 does not play any role in Ca^{2+} -calmodulin modulation of heteromeric channels. Instead, by comparing properties of native channels with heterologously expressed heteromeric channels, the

modulatory subunits CNGA4 and CNGB1b have been shown to be responsible for the physiological modulation of Ca^{2+} -calmodulin. Bradley et al. [44,52] and Munger et al. [37] measured, in excised patches containing native heteromeric olfactory CNG channels, a fast current inhibition upon addition of Ca^{2+} -calmodulin that persisted for several seconds also after calmodulin was removed in Ca^{2+} -free solution. Fig. 3D illustrates the rapid Ca^{2+} -calmodulin inhibition and slower recovery when native olfactory CNG channels were activated by $10 \mu\text{M}$ cAMP in an excised inside-out patch from the knob/cilia of a mouse olfactory sensory neuron.

The modulatory subunits CNGA4 and CNGB1b are necessary for the rapid inhibitory effect of Ca^{2+} -calmodulin and for maintaining the inhibitory action for several seconds, even after Ca^{2+} -calmodulin removal [37,44,52]. Instead, modulation of the rod and cone channels by Ca^{2+} -calmodulin requires only the CNGB1a and CNGB3 subunits, respectively [53,54]. In rods, the mechanism of Ca^{2+} -calmodulin inhibition of the

CNG channel seems to be quite well understood: Ca^{2+} -calmodulin binds to a IQ-type calmodulin binding site in the N-terminal region of the CNGB1a subunit that is also necessary for the interaction with the CNGA1 subunit, thus preventing the direct interaction between the C-terminal region of CNGA1 with the N-terminal region of CNGB1a. Since the interaction between these intracellular channel domains is responsible for a higher ligand sensitivity of the rod channel, the binding of Ca^{2+} -calmodulin decreases the ligand sensitivity causing an inhibitory effect on the rod channel [53,54]. Also the olfactory modulatory subunits have calmodulin binding sites: CNGA4 has a IQ-type calmodulin binding site located at the C-terminal region, while CNGB1b has a similar IQ-type site located at the N-terminal region and a Baa motif in the C-terminal region. It has been shown that the IQ-type sites are necessary and sufficient for Ca^{2+} -calmodulin channel inhibition, whereas the Baa-type site is not necessary [44,55]. However, at present, the molecular mechanism by which the binding of

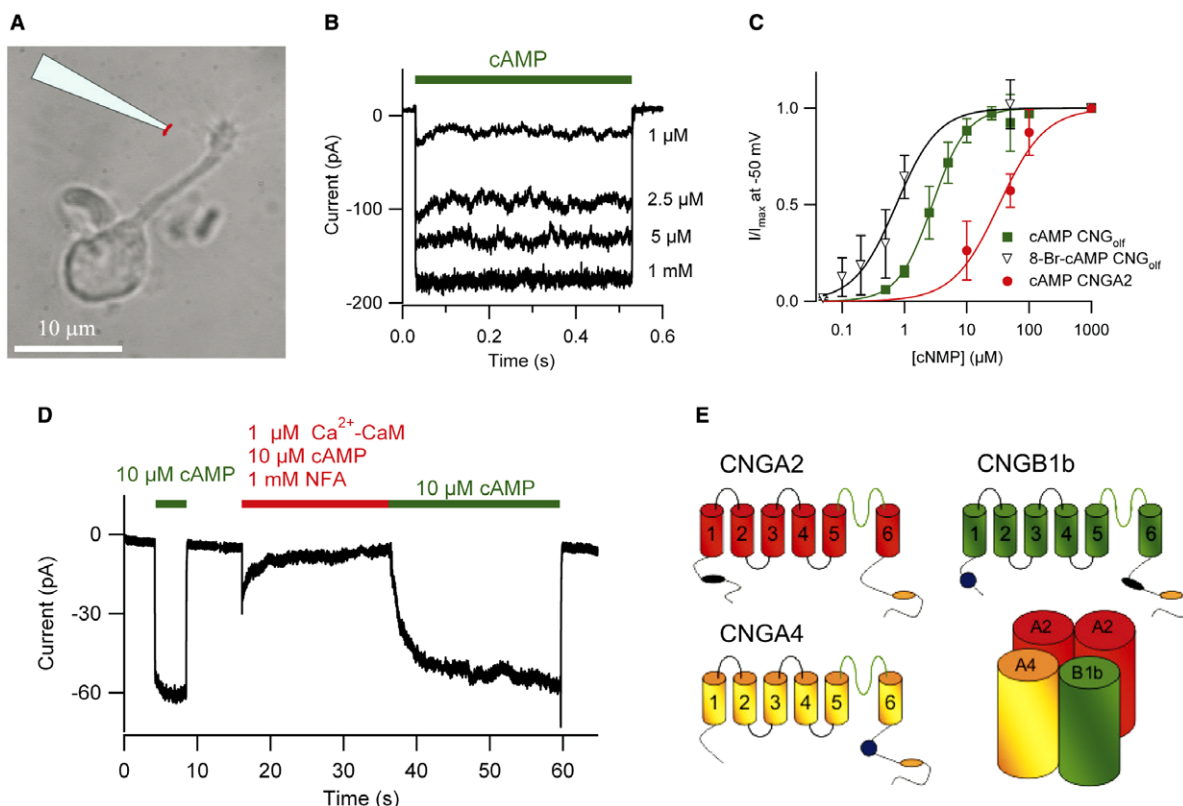


Fig. 3. CNG channels in olfactory sensory neurons. (A) A membrane patch was excised in the inside-out configuration from the dendritic knob/cilia of an isolated mouse olfactory sensory neuron and exposed to different solutions containing cyclic nucleotides to activate the CNG channel. (B) Native olfactory CNG channels were activated in the same patch by the indicated concentrations of cAMP in the absence of Ca^{2+} and Mg^{2+} , in an inside-out excised patch from the knob/cilia. Holding potential was -50 mV. (C) Dose-responses from membrane patches containing native channels activated by cAMP (\square) or by the hydrolysis resistant analogue 8-Br-cAMP (\triangle). Dose-response from patches excised from the membrane of HEK293 cells expressing homomeric CNGA2 channels activated by cAMP (\circ). The continuous lines are the best fit of the Hill equation: $I/I_{\max} = c^n / (c^n + K_{1/2}^n)$ to the data with the following values at -50 mV for the native channel: $K_{1/2}(\text{cAMP}) = 2.7 \mu\text{M}$, $n(\text{cAMP}) = 1.5$, $K_{1/2}(8\text{-Br-cAMP}) = 0.7 \mu\text{M}$, $n(8\text{-Br-cAMP}) = 1.6$. For homomeric CNGA2 channels (\circ) $K_{1/2}(\text{cAMP}) = 33 \mu\text{M}$, $n(\text{cAMP}) = 1.2$. Fits of the Hill equation to data for native channels at $+50$ mV gave: $K_{1/2}(\text{cAMP}) = 1.6 \mu\text{M}$, $n(\text{cAMP}) = 1.4$, $K_{1/2}(8\text{-Br-cAMP}) = 0.4 \mu\text{M}$, $n(8\text{-Br-cAMP}) = 1.5$. (D) Native olfactory CNG channels are inhibited by Ca^{2+} -calmodulin in excised inside-out patches. A patch was exposed to $10 \mu\text{M}$ cAMP in a solution containing nominally 0Ca^{2+} . Then the same patch was exposed to a solution containing, in addition to $10 \mu\text{M}$ cAMP, $1 \mu\text{M}$ calmodulin and $67 \mu\text{M}$ Ca^{2+} (in the presence of 1mM NFA to block native Ca^{2+} -activated Cl current). The addition of Ca^{2+} -calmodulin quickly inhibited the cAMP-gated current that slowly recovered to its initial value after removal of Ca^{2+} -calmodulin (Pifferi, unpublished data). (E) Topological model and assembly of subunits of the olfactory CNG channel. Each transmembrane domain is indicated by a number, the pore loop is located between 5 and 6. The cyclic nucleotide binding site (brown) is located in the C-terminal domain. Calmodulin binding sites of the calcium-dependent 'Baa type' are represented in black, whereas the calcium independent 'IQ-type' are in blue.

Ca^{2+} -calmodulin decreases the ligand sensitivity of the olfactory channel is not yet understood.

5. Channelopathies

Some mutations in the genes encoding CNG subunits have been shown to produce visual disorders in human patients. Some forms of retinitis pigmentosa, an eye disease characterized by a progressive degeneration of the retina which affects night vision and peripheral vision, possibly leading to blindness, are caused by mutations in the CNGA1 or CNGB1 genes of the rod photoreceptors [56]. Achromatopsia, a retinal disorder characterized by the loss of color discrimination and by photophobia, is caused by mutations in the CNGA3 and CNGB3 genes of the cone photoreceptors [57,58]. More than 40 mutations in the CNGA3 channel gene, giving rise to various forms of achromatopsia, have been identified [59]. Peng et al. [60] functionally characterized some mutations in the human CNGB3 subunit and reported alterations in cyclic nucleotide sensitivity, ion selectivity, single channel properties, and plasma membrane targeting of the heteromeric CNG cone channel.

The function of CNG channel subunits has been also investigated by targeted disruption of genes encoding the CNGA2, A3, A4 or B1 subunits [37,60–64]. Deletion of the CNGA3 subunit produced mice lacking cone responses to light, whereas the rod pathway was completely intact [63]. Knock-out of the CNGB1 gene in mice caused the alteration in targeting of the CNGA1 subunit in rod outer segment, abolished the response to light and caused retinal degeneration, similarly to human retinitis pigmentosa [61]. Knock-out of the olfactory CNGA2 gene caused the absence of any detectable response to odorants in mice, that therefore had a general anosmia, and demonstrated that the cAMP-mediated pathway is the only transduction mechanism that mediates odorant detection [64]. Knock-out of the olfactory CNGA4 gene caused alterations in odorant adaptation, furtherly demonstrating the fundamental role of modulatory subunits in the physiological function of CNG channels [37].

6. Conclusions

The recent progress in our knowledge of the biophysical and physiological properties of CNG channels allows a better understanding of sensory transduction. However, up to today, a few questions are still open. It has been shown that the Ca^{2+} -dependent regulation of CNG channels in olfactory sensory is responsible for adaptation and that the ligand sensitivity is reduced when Ca^{2+} -calmodulin interacts with both modulatory subunits [37,44,52,55], but the nature of this interaction is not yet understood.

On the contrary, in the retinal rod channel, it has been shown that the binding of Ca^{2+} -calmodulin to the CNGB1a N-terminus decreases the ligand sensitivity by interfering with the direct interaction with the CNGA1 C-terminus, an inter-domain interaction, essential to have high ligand sensitivity [51]. However, the physiological role of Ca^{2+} -calmodulin regulation in retinal rods is unclear and it does not seem to be involved in adaptation [65,66].

It has been proposed that the endogenous factor co-assembled with the native olfactory channel is calmodulin, although

a conclusive demonstration is still lacking. Moreover, some experimental evidence argues against this hypothesis, in particular the endogenous factor appears to bind the CNG channels in a very stable manner, being washed away only after intense rinsing in Ca^{2+} -free solution [40,43,44]. However, it is also possible to speculate that the binding of “native” calmodulin is more stable because the channel or the calmodulin itself undergoes post-transductional modifications that change the properties of the interaction. On the other hand, it cannot be excluded that also other proteins, in addition to calmodulin, contribute to the Ca^{2+} -mediated modulation of olfactory CNG channels.

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