Int J Physiol Pathophysiol Pharmacol 2016;8(3):95-108
www.ijppp.org /ISSN:1944-8171/IJPPP0034003

Review Article

Type 3 adenylyl cyclase: a key enzyme mediating the cAMP signaling in neuronal cilia

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Received June 19, 2016; Accepted September 6, 2016; Epub September 22, 2016; Published September 30, 2016

Abstract: Cilia are rigid, centriole-derived, microtubule-based organelles present in a majority of vertebrate cells including neurons. They are considered the cellular "antennae" attuned for detecting a range of extracellular signals including photons, odorants, morphogens, hormones and mechanical forces. The ciliary microenvironment is distinct from most actin-based subcellular structures such as microvilli or synapses. In the nervous system, there is no evidence that neuronal cilia process any synaptic structure. Apparently, the structural features of neuronal cilia do not allow them to harbor any synaptic connections. Nevertheless, a large number of G protein-coupled receptors (GPCRs) including odorant receptors, rhodopsin, Smoothened, and type 6 serotonin receptor are found in cilia, suggesting that these tiny processes largely depend on metabotropic receptors and their tuned signals to impact neuronal functions. The type 3 adenylyl cyclase (AC3), widely known as a cilia marker, is highly and predominantly expressed in olfactory sensory cilia and primary cilia throughout the brain. We discovered that ablation of AC3 in mice leads to pleiotropic phenotypes including anosmia, failure to detect mechanical stimulation of airflow, cognitive deficit, obesity, and depression-like behaviors. Multiple lines of human genetic evidence also demonstrate that AC3 is associated with obesity, major depressive disorder (MDD), sarcoidosis, and infertility, underscoring its functional importance. Here we review recent progress on AC3, a key enzyme mediating the cAMP signaling in neuronal cilia.

Keywords: AC3, olfaction, obesity, depression, primary cilia, mechanosensation, cAMP signaling

Introduction

During the course of evolution, cilia have become very diverse [1]. Cilia can be found in ancestral unicellular eukaryotic organisms such as protozoan paramecium and algae. Most vertebrate cells possess a single primary (solitary) cilium [2], while only limited cell types of invertebrates such as sensory neurons or sperms contain cilia [3]. Some cell types in vertebrates such as respiratory epithelial cells, ependymal cells, and olfactory sensory neurons possess multiple cilia. All types of cilia emanate from the basal body and protrude from the plasma membrane. For primary cilia, the basal body residing underneath the plasma membrane is a special form of the mother centriole when a cell stays in its quiescent G0 phase[2, 4]. Because the centriole is a microtubule-based protein complex, cilia have microtubule axoneme cores that are made up of at least nine sets of microtubule doublets (**Figure 1**) [4]. Traditionally, cilia are classified as motile cilia or immotile cilia. But according to Takeda and Narita [1], the diversity of cilia/flagella can more strictly be classified into 8 different categories, including solitary 9 + 0 non-motile cilia (authentic primary cilia, see **Figure 1**), solitary 9 + 2 motile cilia (flagella of sperm), multiple 9 + 2 non-motile cilia (olfactory cilia), and multiple 9 + 2 motile cilia (respiratory motile cilia), as well as 4 other types. The ciliary compartment is tiny (**Figure 1**) and separated from cytosolic environments by the transition zone at the base [5, 6]. No membrane vesicles are found in the ciliary compartment and likewise no proteins are synthesized there. A sieve-like diffusion barrier in the transition zone [7] is formed by septin 2, a member of the septin family of guanosine triphosphatases [8]. The diffusion barrier functions to prevent large particles from diffusing into or out of the ciliary compartment [8].

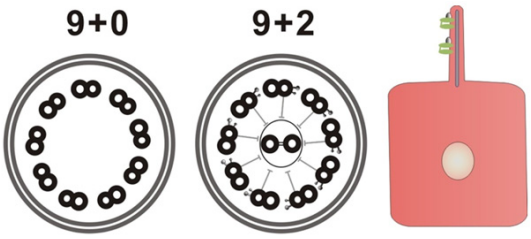

Figure 1. Ciliary structure. Schematic showing cross section of cilia revealing the 9 + 0 and 9 + 2 arrangement of nine peripheral microtubule doublets at the axoneme. Right, diagrammatic drawing of cilia structure of in cells. Primary cilium locates on the soma in the proximity of the nucleus and most of them contain a 9 + 0 structure.

Consequently, cilia depend on a unique complex of motor and adaptor proteins (coined intraflagellar transport, IFT) for protein transportation [9]. Large ciliary proteins (with a diameter > 7.9 nm) have to be transported into and out of cilia by the IFT system [7, 10], which is essential for cilium formation and maintenance [9].

Recently, many ion channels [11, 12], a large variety of receptors including GPCRs [13], and enzymes as well as their downstream effectors, have been identified in primary cilia [2]. For example, the Sonic hedgehog signaling pathway requires primary cilia to transduce morphogenic signals [14]. Thus, malfunctions of primary cilia lead to various developmental defects. In the last decade, primary cilia have emerged as an important research field with great potential and broad therapeutic implications. They exert a broad spectrum of physiological functions varying from cellular locomotion, renal function, sensations and development to energy balance and mechanosensation. Defects in cilia lead to a range of diseases that include sensory defects, obesity, diabetes, infertility, cystic kidney disease, liver disease, developmental abnormalities, intellectual disability and mental disorders (collectively coined ciliopathies) [2, 3, 11, 12, 15-19]. Cilia can be visualized by immunostaining protein markers including type 3 somatostatin receptor, acetylated α-tubulin, Arf13b, and AC3. Among these protein markers, AC3 is expressed almost exclusively in olfactory sensory cilia or primary cilia [20]. This review discusses our current understanding of AC3 and the role of cyclic adenosine 3', 5'-monophosphate (cAMP) signaling in neuronal cilia.

Adenyly cyclases and AC3

cAMP regulates a large number of physiological functions including fertilization, development, gene expression, sensory function, learning and memory, smooth muscle contraction, heart beat, and hormone secretion [21]. cAMP-mediated signaling is one of the most important and ubiquitously distributed signaling pathways in the phyla. cAMP is synthesized by adenylyl cyclases (ACs), which catalyze the conversion of adenine triphosphate (ATP) into cAMP in response to a wide range of extracellular and intracellular signals. In mammals, there are nine membrane-associated ACs and one water soluble adenylyl cyclase (soluble AC). Mem brane-associated ACs have about 1,028-1,248 amino acids, which cross the plasma membrane 12 times, forming 2 cassettes of 6 transmembrane-spanning domains followed by two large cytosolic domains [22]. These two cassettes are domains comprised of two cyclic structures designated C1 and C2, which are binding sites for forskolin, and G-proteins, and comprise the active sites of the ACs. Each AC has a unique primary sequence which leads to a variety of AC regulatory mechanisms [23]. Calcium and G-protein coupled receptors (GPCR) are the major regulators of adenylyl cyclase in the nervous system [24, 25]. The membrane associated ACs can be further categorized into four different classes. Class I (AC1, AC3, and AC8) is calcium/calmodulin-sensitive forms. The major calcium-stimulated forms of ACs include AC1 and AC8, which are very important for long-term memory formation [24, 26], while AC3 is inhibited by calcium [27, 28]. Class II (AC2, AC4 and AC7) is Gβγ-stimulatory forms and are insensitive to calcium stimulation. Class III (AC5 and AC6) is inhibited by $G\alpha_i$ isoforms [29]. Class IV is AC9, which is distantly related to other membrane-bound ACs and cannot be activated by calcium [30]. All membrane-associated ACs, except for AC9 [30], are stimulated by forskolin. All membrane-bound ACs are found to be expressed in the central nervous system (CNS) [21, 31], although their regional distributions in the brain vary markedly [32]. Soluble AC does not have any membrane-associated structure, but retains the C1 and C2 enzymatic domains. It is predominantly expressed in sperm but also identified in other tissues in low abundance [33]. Soluble AC cannot be stimulated by hor-

mones, G proteins, or forskolin, but it can be activated by bicarbonate and intracellular calcium [34]. Soluble AC plays an essential role for sperm motility, capacitation and fertilization [33, 35].

AC3 is special among the membrane-associated ACs due to its predominant expression in cilia. An AC3 cDNA clone was originally detected in a rat olfactory cDNA library and northern analysis using total RNA in 1990. It was originally shown that the expression of AC3's mRNA is limited to the olfactory epithelium [36]. AC3 was thereby initially thought to be an olfactory-specific adenylyl cyclase. However, it was discovered two years later by Xia et al. that AC3 is not specific to olfactory sensory neurons and that mRNA of AC3 is present in many tissue types including brain, spinal cord, adrenal medulla, adrenal cortex, heart atrium, aorta, lung, retina, 293 cells and PC-12 cells [37]. Despite this discovery, studies on AC3 mostly focused on olfactory sensory neurons for 15 years. It was not recognized that AC3 is also expressed in neuronal primary cilia until an effective anti-AC3 antibody (sc-588, Santa Cruz) for immunostaining was commercially available. Using the AC3 antibody in an immunohistochemical assay, Bishop et al. showed that AC3 predominantly localizes to neuronal primary cilia throughout the adult mouse brain including the cortex, hippocampus, hypothalamus, amygdala, nucleus accumbens, and dorsal raphe nucleus [20]. AC3 proteins are also present in the primary cilia of astrocytes [20] and of epithelial cells of the choroid plexus in the adult brain [38]. AC3 protein expression is not limited to the brain. For example, AC3 protein has been found to be expressed in primary cilia of kidneys [39], the pancreas [40], and brown and white adipose tissue [41]. In addition, AC3 expression were detected in tumors [42], vascular [43] and bronchial smooth muscle [44], male germ cells [45], and hepatic cells [46], although its ciliary location has not been clarified in these reports.

In addition to AC3, other types of ACs have been reported to be expressed in primary cilia. AC5/6 were observed in non-neuronal primary cilia, and AC6 is found to be in primary cilia of bone cells [47]. AC6 and cAMP are thought to mediate primary cilia-dependent mechanosensation [47] and play a role in loading-induced bone adaptation [48]. AC5/6 as well as AC3 have been found to be expressed in primary cilia of cerebellar granular neuron precursors (CGNPs) and regulate the hedgehog pathway [49]. However, apparently AC3 is more predominantly enriched in primary cilia than AC5/6 [49] and AC5/6 have strong distribution in other subcellular locations. AC5/6 (as well as AC3) were also detected in primary cilia of renal epithelial cells [50]. It has been reported that AC4, AC6, and AC8 are expressed in cholangiocyte primary cilia, although they are also highly distributed to other subcellular locations [51]. Calcium-stimulated AC8 is found to be present in neuronal primary cilia of the hippocampus and co-localizes with β2-adrenergic receptor [52]. AC2 and AC4 have been shown to be present in olfactory cilia, but they have no function on olfactory perception because they do not compensate the loss of function of AC3 knockout mice in anosmia [53]. The functions of these ACs in cilia remain to be elucidated.

AC3 is essential for olfactory cAMP signal transduction

Olfactory cilia are the primary sensory organelles for olfaction in the main olfactory epithelia and are located at the knobs of olfactory sensory neurons. Odorant signal transduction is initiated by the binding of odorants to olfactory receptors in olfactory cilia, which activate the associated heterotrimeric GTP-binding G protein [54], G_{olf} protein. Once activated, G_{α} subunit of the G_{olf} protein exchanges guanosine diphosphate (GDP) for guanosine triphosphate (GTP), and dissociates from the $G_{\beta/\gamma}$ complex to stimulate AC3 activity [53]. The cAMP generated by AC3 binds to and activates cyclic nucleotide–gated (CNG) channels [55], resulting in an influx of Na^+ and Ca^{2+} ions and ultimately leading to initiation of action potentials in olfactory sensory neurons [56]. The olfactory signals are then converted into electrical signals that are sent to the olfactory bulb for information integration. Proteins essential for olfactory signal transduction, including olfactory receptors, G_{olf} -protein, AC3 and CNG channels, are all found to be highly enriched at olfactory cilia of olfactory sensory neurons [57]. In mice, there are about one thousand different odorant receptor genes for detecting different odorants [56]; humans have about 300 different receptor genes [56, 57]. AC3 is the only functional ade-

nylyl cyclase expressed in olfactory cilia, meaning that hundreds of different odor receptors rely on AC3 to transmit olfactory signals. Therefore, AC3 and cAMP signaling are obligate components mediating the olfactory signal transduction in olfactory cilia. Consequently, knocking out the gene for AC3 leads to almost complete loss of smell [53]. In addition, as chemosensory signals generated by mouse pups trigger maternal behavior in females, which is partly mediated by olfactory sensory neurons in the main olfactory epithelia, the female's maternal behaviors are impaired after ablation of AC3 [58].

Cilia detect mechanical force

In nature, a wealth of cilia can generate mechanical force. These include sperm and algal flagella, as well as cilia of paramecium, cilia of respiratory epithelia, cilia in oviduct, cilia of ependymal cells, and embryonic nodal cilia. However, cilia not only generate mechanical force, but also sense pressure or mechanical force [59-61]. Microtubules are rigid cytoskeletal filaments, and their mechanics [62] and the axonemal structure confers cilia with a certain rigidity [63]. Moreover, cilia have a diameter of 200-300 nm, which is mostly occupied by the microtubular axoneme core enwreathed by a thin layer of plasma membrane. Cilia thereby have limited intracellular space to accommodate soluble proteins. Most of the ciliary proteins are associated either with the ciliary membrane or with the microtubule anoneme core. In addition, cilia protrude from the plasma membrane, like antennae, which is spatially optimal for detection. For these reasons, microtubule-based cilia are structurally suited to detect mechanical force, and sensing mechanical force could be a common feature for various cilia of many cell types.

Indeed, mechanosensing cilia in vertebrates include renal, chondrocyte and endothelial cell primary cilia as well as embryonic nodal cilia, among others. Mechanosensing cilia in ciliated neurons of *C. elegans* [64] and *Drosophila* are responsible for touch [65, 66] and hearing [67]. Some motile cilia can simultaneously detect mechanical force [61]. The most extensively studied mechanosensitivity case is renal cilia. This is because defects in renal cilia are associated with polycystic kidney disease, the most common hereditary disease in humans. Primary cilia in the apical surface of the epithelial layer of the nephron were once thought to sense the mechanical pressure of urine flow. This sensation of mechanical force by cilia of epithelial cells in the kidneys was considered to be crucial for the normal maintenance of renal physiology, and failure to detect the mechanical force of urine flow was postulated to cause polysystic kidney disease [4, 47]. However, Freedman et al. have showed that kidney organoids derived from ADPKD pluripotent epiblast spheroids form cysts more frequently those from normal patients in the absence of liquid flow [68]. This is contradictory evidence auguring against that mechanical force is involved in cyst formation [68]. Moreover, it has been hypothesized that intracellular calcium signaling mediates the signal transduction of mechanical force. However, Clapham lab has recently challenged the calcium source of mechnosensation of renal cilia. Using a calcium imaging technique, they showed that the proposed cilia-origin of the calcium change is an artifact caused by the lack of continuity of the cell body and cilia in the focal plane [69]. Therefore, it is not yet resolved which intracellular signaling pathway mediates the signal transduction of renal cilia's mechanosensitivity and how important it is for cyst formation.

AC3 mediates the signal pathway of mechanosensation for airflow

Olfaction starts with a sniff. Sniffing modulates olfactory perception by a number of ways [70-72], including regulation of the olfactory detection threshold [73] and facilitation of discrimination of odorants [74]. It has been discovered that sniffing clean air without odorants can activate the human olfactory cortex and other regions of brain [75, 76]. Air-puffs through the nostrils activate the amygdala in monkeys [77] and also cause neuronal firing in the MOB of mice [78]. These studies suggest the possibility that the airflow of sniffing per se may exert a mechanical force directly on olfactory cilia to activate olfactory sensory neurons. Indeed, we discovered that olfactory sensory neurons not only detect the chemical signals of odorants but also detect the mechanical force of airflow. We used a technique called electro-olfactogram (EOG) recording to establish the mechanosensitivity of olfactory sensory neurons in response to airflow (**Figure 2**). EOG measures the field potential of main olfactory epithelia

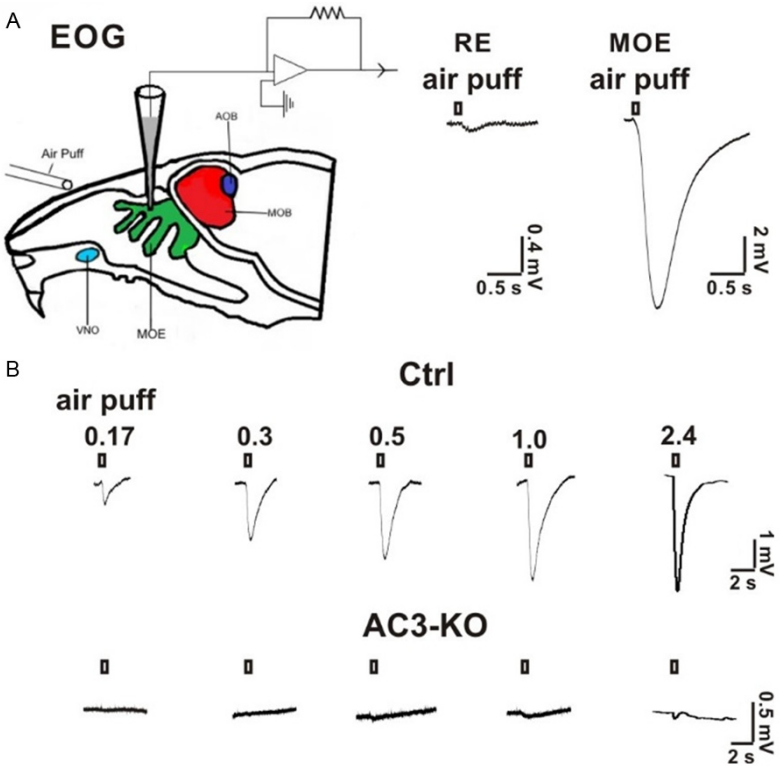

Figure 2. Olfactory cilia sense the mechanical stimulation of air flow. A. Left, a configuration of EOG recording. Right, air puff of clear nitrogen elicits electrical response in main olfactory epithelia (MOE), but not in respiratory epithelia (RE). B. MOE from AC3 KO mice are insensitive to airflow. Various air flowrates (L/min) have been applied onto the MOE. EOG recording traces are modified from Chen et al., J. Neurosci, 2012 [79]. EOG, electro-olfactogram; MOB, main olfactory bulb; AOB, accessory olfactory bulb; VNO, vomeronnasal organ.

responding to an air puff of odorants. We performed EOG recording in an isolated olfactory epithelium. We found that air puffs of pure nitrogen, clear air without odorants, can evoke a pronounced field potential in olfactory epithelia, but not in respiratory epithelia in the nasal cavity (**Figure 2**). This indicates that olfactory epithelia can respond to the mechanical stimulation of airflow. We then examined which signaling pathway mediates this response. We used forskolin to first activate adenylyl cyclase to make cAMP, which will activate and subsequently desensitize the olfactory signal pathway. We found that application of forskolin strongly inhibits or desensitizes the airflow-stimulated EOG responses, while the addition of vehicle has no effect, suggesting that adenylyl cyclase is essential for the airflow-sensitive response. Moreover, the airflow-sensitive EOG response of main olfactory epithelia can be inhibited by SCH202676, a general inhibitor of G-protein coupled receptors [79] and desensitized by odorant mix. Importantly, we also tested the effect of airflow on EOG response using AC3 knockout (KO) mice. Air puffs to the main olfactory epithelia generated strong EOG responses in the main olfactory epithelia of AC3 wild type (WT) mice, which are dose-dependent, but not AC3 KO mice (**Figure 2**). These data indicate that AC3 is required for sensing the mechanostimulation of airflow.

It is well established that airflow from respiration or sniffing causes a rhythmic oscillation in olfactory sensory neurons and the olfactory bulb [70]. Our study provides a reasonable mechanistic explanation for such rhythmic oscillation. It is worth mentioning that airflow stimulation of olfactory sensory neurons falls within the physiological range of mice: the estimate flow rate of sniffing in mice ranges from 0.03-0.18 l/min [80] and we have determined the threshold for airflow activation and found that threshold for airflow response was between 0.03-0.06 l/min [79]. Thus, the airflow stimulation of olfactory cilia is within the physiological sniffing range of mice. Although the absolute value of the airflow-stimulated response in olfactory sensory neurons is not very strong [79], it can still affect the membrane potential and facilitate the depolarization of olfactory sensory neurons, thereby promoting initiation of action potentials. Physiologically, olfactory sensory neurons should not be too sensitive to airflow. Otherwise, it could increase noise during olfactory perception and interfere with the coding of odor information.

Our study is also in line with the original reports by Ma and colleagues. Using patch clamp whole-cell recording in acute olfactory tissue slices, Ma and colleagues have discovered that olfactory cilia of some olfactory sensory neurons in the main olfactory cilia or in the septum

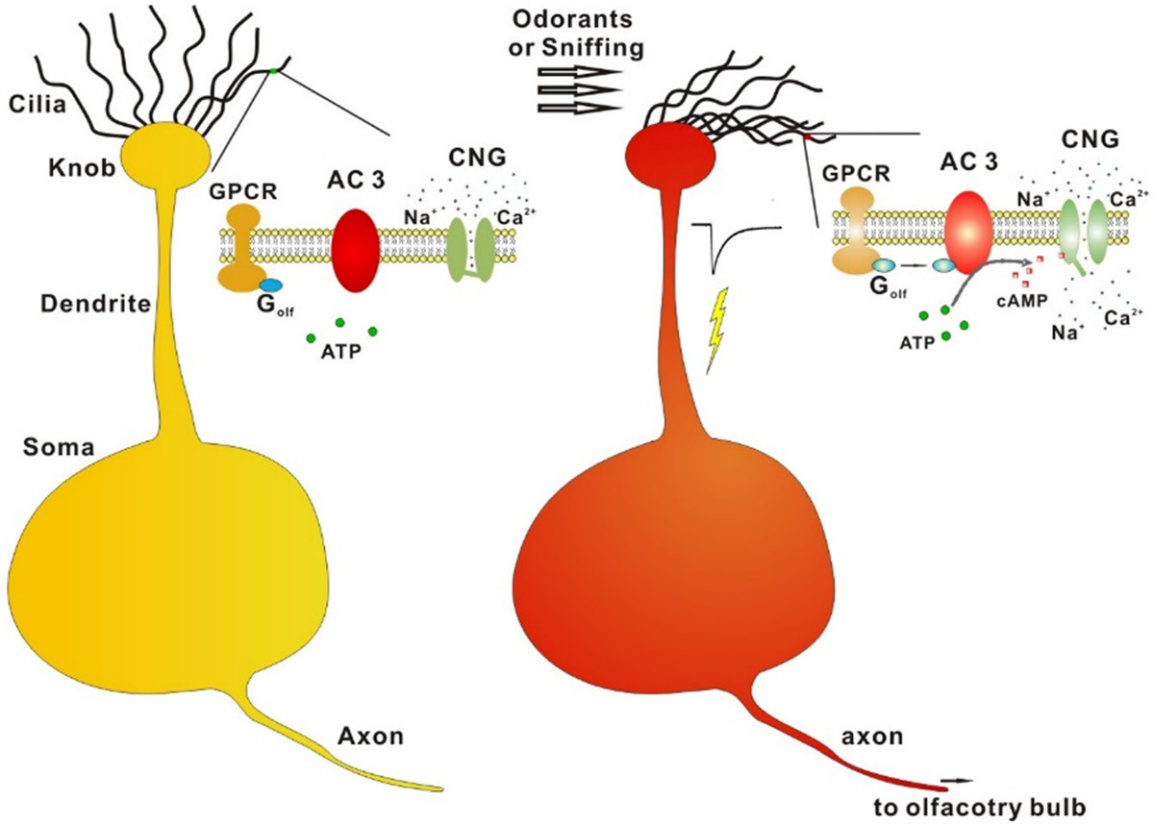

Figure 3. Mechanosensation of olfactory sensing neurons shares a common signaling pathway with odorant perception. Airflow (or odorants) activates olfactory receptors in olfactory cilia, which stimulate G_{olf} and in turn activate AC3 to produce cAMP. cAMP subsequently opens CNG channels, leading to cation influx and activation of olfactory sensing neuron.

organ can sense a mechanical force generated by a stream of liquid [81]. Ma and colleagues also elegantly demonstrated that some types, albeit not all, of odorant receptors [82] and the CNG channels [81] mediate the mechanosensitivity to olfactory sensory neurons. In addition, they also provided similar evidence that AC3 is required for transducing this mechanosensitive signal [82]. All together, these lines of evidence have established that olfactory sensory neurons can sense the mechanical force of airflow and that the mechanosensation of olfactory cilia shares the same cAMP signaling mechanism as chemosensation (see **Figure 3**). These studies also suggest that the airflow of sniffing can increase the sensitivity of odorant detection [83] through synergistically stimulating olfactory sensory neurons.

AC3 represents a key enzyme for cAMP signaling in primary cilia in the CNS

In the CNS, virtually every neuron has a solitary primary cilium. It is fairly short (2-12 μm) compared to neuronal dendrites and axons. However, neuronal primary cilia occupy good location and they are located on the soma, in the proximity of the nucleus. Moreover, primary cilia of matured neurons are less plastic than synapses and apparently reside on the neuronal soma permanently. Neuronal primary cilia do not have synaptic structures or connections. Neither ionotropic glutamate receptors nor $GABA_A$ receptors have been identified on neuronal primary cilia. Therefore, this tiny organelle depends on metabotropic receptors rather than synaptic inputs to influence neuronal function. Notably, two major metabotropic receptor-dependent signaling pathways function in neuronal primary cilia: Sonic hedgehog (SHH) [84, 85] and cAMP signaling pathways [86]. SHH signaling is known to regulate neuronal development and the formation of adult neural stem cells [87, 88]. A number of ciliary GPCRs including type 6 serotonin receptor (5-HT6) [89], type 2 neuropeptide Y receptor (NYP2R) [90], type 2/3 galanin receptor (GALR2/3) [90], type 1

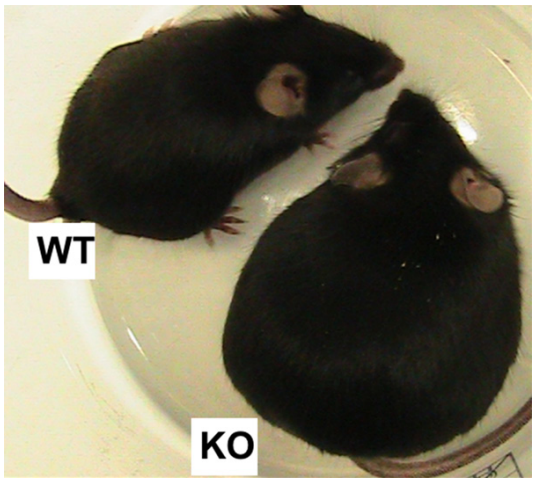

Figure 4. AC3 knockout mice are obese.

melanin concentrating hormone receptor (MCH1) [91], and type 3 somatostatin receptor (SST3) [89] are $G\alpha_s$- or $G\alpha_i$- coupled receptors that rely on adenylyl cyclase to transduce cAMP signal into the neuron. Among the 9 membrane-associated ACs in mammals, AC3 is a major adenylyl cyclase and is highly enriched in neuronal primary cilia [20], with negligible expression in other subcellular locations (observations based on AC3 WT and KO mice). For these reasons, AC3 is a "master" enzyme to mediate cAMP signaling in neuronal cilia and is crucial for the neuronal "antenna" to execute its functions in the CNS.

AC3 is associated with both obesity and major depression

AC3 in neuronal primary cilia also has important roles in the CNS. One important human health impact is on energy balance [92]. Obesity is one of the most common symptoms for a variety of ciliopathies including Bardet-Biedl Syndrome [15, 93, 94]. Several human genetic analyses have clearly defined *ADCY3* as a gene associated with obesity [95-99]. We discovered that conventional AC3 KO mice exhibit adult-onset obesity (**Figure 4**) [41]. This transgenic strain exhibits hyperphagia, hyperinsulinemia, and increased serum leptin [41]. Conversely, a gain-of-function mutation of AC3 in mice can protect the animals from diet-induced obesity [100]. Moreover, we have found that conditional AC3 tamoxifen-inducible KO mice are hyperphagic and obese (unpublished observations), suggesting that the obesity phenotype is not a secondary effect caused by developmental abnormality. It is very striking that ablations of ciliary proteins including KIF3a [101], Bbip10 [90], IFT88 [102], Tubby [103], BBS1, and BBS4 [93, 102] all lead to obesity in mouse models. We further found that ablation of AC3 specifically in the hypothalamus caused hyperphagia and obesity (unpublished observations). This evidence suggests that primary cilia regulates energy balance and AC3 in primary cilia in the hypothalamus is involved in this regulation. Nevertheless, it is unclear if ciliary cAMP signaling cross talks with the leptin-mediated signaling pathway in the hypothalamus. It is tempting to postulate that AC3 may functionally couple to melanocortin 4 receptor (MC4R) in the hypothalamus, because activation of adenylyl cyclase activity by alpha-melanocyte stimulating hormone (α-MSH) downstream in the leptin pathway is required for the anorectic activity of leptin. Since transgenic mice lacking MC4R and AC3 KO mice exhibit similar phenotypes including obesity, hyperphasia, and hyperinsulinemia [41, 104], it is possible that MC4R receptors may couple to stimulation of AC3 in neurons of the paraventricular nucleus of hypothalamus (PVH) to generate cAMP signals which lead to appetite suppression and/or energy utilization.

Another health impact of AC3 pertains to on major depressive disorder (MDD). Human major depression is hereditary but has low heritability compared with other major psychiatric disorders such as schizophrenia and bipolar disorders [105]. However, a recent study based on over 5 thousand patients with MDD and healthy subjects has implicated cAMP signaling in MDD and identified AC3 (*ADCY3)* as a top-ranked gene relevant for MDD [106]. Consistently, a large number of studies have implicated AC activity in depression [107, 108]. Current antidepressants have the potential to indirectly stimulate AC activity [109]. Platelet adenylyl cyclase activity, which is thought to be mainly AC3 [110], has been proposed as a biological marker for MDD [111] because patients with a history of depression have lower mean levels of platelet cAMP [112] than control human subjects. In addition, depressed patients have a reduced sense of smell [113]; the severity of depression is correlated with decreased sensitivity of smell [114].

We first discovered that AC3 conventional KO mice exhibit strong depression-like phenotypes. AC3 conventional KO mice exhibit strong depression-like phenotypes in several behavioral assays including tail-suspension test, novelty suppressed feeding test and nesting behavioral test. Disturbances of sleep including alterations in sleep architecture and increased rapid eye movement (REM) sleep are typical for MDD patients and are one of the core symptoms associated with MDD. Therefore, the sleep architecture of AC3 KO mice was analyzed by electroencephalography/electromyography (EEG/EMG) recordings, EEG-EMG analysis showed that AC3 KO mice have altered sleep patterns characterized by an increased percentage of rapid eye movement sleep. AC3 KO mice also show neuronal atrophy, consistent with its role in cortical morphorgenesis [89, 115]. Furthermore, we found that basal synaptic activity at CA3-CA1 synapses was significantly lower in AC3 KO mice, and they also exhibited attenuated long-term potentiation as well as deficits in spatial navigation. To rule out that these defects are secondary responses to anosmia or developmental defects, we generated a conditional AC3 floxed mouse strain. Afterwards, we crossed AC3 floxed mice with a forebrain-specific Cre recombinase mouse strain to inactivate AC3 function selectively in the forebrain. We also bred the AC3 floxed mice with UBC-Cre/ERT2 mice to inducibly ablate AC3 in adult mice. We observed that both AC3 forebrain-specific and AC3-inducible knockout mice exhibited pro-depression phenotypes without anosmia[116]. Together, the evidence from human studies and our animal study strongly substantiate that AC3 is a genetic risk factor for major depression.

The functional mechanism of AC3 in neuronal primary cilia in the CNS is unknown

Although AC3 is strongly associated with obesity and depression, it is unclear how AC3 modulates neuronal function in the CNS. In the peripheral nervous system (PNS), sensory cilia can directly control neuronal excitation (or inhibition), which is essential for several types of sensory perception. For example, mechanosensing cilia in ciliated neurons of *C. elegans* and Drosophila are required for touch and hearing, respectively [117, 118]. In mammals, the outer segments of retinal cones or rods are specialized primary cilia controlling membrane potential of cones or rods, transmitting vision signals [119]. Moreover, olfactory cilia directly govern excitation of olfactory sensory neurons, mediating olfaction. As mentioned above, it is AC3 in olfactory cilia that transmits excitatory signals from upstream odorant receptors to downstream cyclic nucleotide-gated ion channels [55]. Olfactory sensory neurons without AC3 are completely silent to odorant stimulation [53]. In contrast, in the CNS, neurons rely on synaptic inputs, rather than ciliary signals, for membrane potential depolarization and action potential initiation after temporal and spatial summation. However, each CNS neuron also possesses a primary cilium on the soma [20], the crucial location for spatial summation of neuronal membrane potential. It raises the possibility that primary cilia may indirectly modulate neuronal membrane potential via a secondary messenger. Because AC3 is predominantly and almost ubiquitously expressed in primary cilia in adult CNS neurons [20], it is plausible that AC3 in primary cilia may indirectly regulate neuronal membrane potential of CNS neurons. It has been shown that AC3 may also modulate morphology of neuronal dendrites [89, 116, 120, 121]. Determination of the signal transduction pathway triggered by cAMP locally in neuronal primary cilia could provide a clear answer to the functional mechanism of AC3 in the brain.

Summary

The rigid, centriole-derived, spatial elongated cilia possess an exquisite microtubule cytoskeleton and unique microenvironment that facilitate chemical and mechanical stimuli to the transduction apparatus. AC3 plays key roles in mediating cAMP signaling in the signal apparatus. In olfactory cilia, AC3 is essential for mediating olfactory signal transduction and mechanical stimulation of airflow. In the CNS, AC3 plays critical roles in regulating a number of physiological functions including energy homeostasis and mood [116]. In addition, AC3 is genetically associated with sarcoidosis [122], and infertility [123]. All highlight the pathophysiological significance of this enzyme. Therefore, further investigation is warranted to understand the molecular mechanism of AC3-mediated cAMP signaling in neuronal primary cilia in the CNS.

Acknowledgements

This study was supported by National Institutes of Health Grants MH073601 (to DRS) and MH105746 (to XC).

Disclosure of conflict of interest

None.

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