the animals (n = 16) for treatment with 700 U of Pal or buffer. Enzyme treatment here completely eliminated pneumococci from five of eight animals and significantly decreased titers in the remaining three (P < 0.001) (Fig. 3B). Each experiment included three uncolonized control animals that revealed no S. pneumoniae. These results indicate that pneumococci on mucosal surfaces are highly susceptible to the action of the lytic enzyme.

We further addressed the question whether surviving pneumococci are able to recolonize the nasopharynx of mice after a single dose of enzyme. Thirty mice were colonized as before (day -2) and randomized 42 hours later for treatment with 1400 U of Pal or buffer. Three mice of each group were killed on days 0, 2, 4, 6, and 8 and the nasal wash was analyzed. All buffer-treated mice remained colonized throughout the experiment (mean log$_{10}$ 3.0) with titers decreasing progressively from day 6. Pal-treated mice had sterile nasal cultures on day 0 (5 hours after treatment), and thereafter, there was a discrete reappearance of pneumococci in the nasopharynx of mice after a single dose of Pal and other pneumococcal phage lytic enzymes is choline, a molecule that is necessary for pneumococcal viability (9, 23, 24). While not yet proven, it is possible that during a phage’s association with bacteria over the millennia, to avoid being trapped inside the host, the binding domain of lytic enzymes has evolved to target a unique and essential molecule in the cell wall, making resistance to these enzymes a rare event.

We believe that with Pal and similar bacteriophage lytic enzymes, we may have the opportunity to control or eliminate nasopharyngeal colonization by S. pneumoniae and thus significantly reduce or prevent infection by these bacteria. Because bacteriophage have been described for nearly all bacteria, this targeted approach to control and/or prevent infection may be applied to other pathogens (particularly Gram-positive bacteria) whose reservoir or site of infection is the human mucous membrane.

**References and Notes**

2. S. I. Pelton, Vaccine 19 (suppl. 1), S96 (2000).

Central Role of the CNGA4 Channel Subunit in Ca$^{2+}$-Calmodulin–Dependent Odor Adaptation

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Heteromultimeric cyclic nucleotide–gated (CNG) channels play a central role in the transduction of odorant signals and subsequent adaptation. The contributions of individual subunits to native channel function in olfactory receptor neurons remain unclear. Here, we show that the targeted deletion of the mouse CNGA4 gene, which encodes a modulatory CNG subunit, results in a defect in odorant-dependent adaptation. Channels in excised membrane patches from the CNGA4 null mouse exhibited slower Ca$^{2+}$–calmodulin-mediated channel desensitization. Thus, the CNGA4 subunit accelerates the Ca$^{2+}$-mediated negative feedback in olfactory signaling and allows rapid adaptation in this sensory system.

Olfactory receptor neurons (ORNs) respond to odorant stimulation with a receptor-mediated increase in intracellular cyclic adenosine 3’,5’-monophosphate (cAMP), which directly activates a cyclic nucleotide–gated (CNG) channel in the plasma membrane (1). Calci-
um ions entering the cell through the open channel, in addition to contributing to the receptor potential (2, 3), mediate cellular adaptation (4, 5). A major mechanism for the rapid adaptation to odors is the Ca\(^{2+}\)–calmodulin (Ca\(^{2+}\)–CaM)–mediated desensitization of the CNG channel (6–10). Three CNG subunits are expressed in ORNs. One of these, CNGA2, is sufficient for generating a cyclic nucleotide-activated conductance and is a target for Ca\(^{2+}\)–CaM–dependent desensitization in a heterologous expression system (6, 7, 11). Two other subunits, CNGA4 and CNGB1b, assemble with CNGA2 in the olfactory epithelium (12) and increase the nucleotide sensitivity of the CNGA2 subunit when coexpressed in vitro (12–15). The contributions of these modulatory subunits to odorant-induced responses in olfactory neurons have not been established.

To define the role of the CNGA4 subunit in native olfactory function, we used gene targeting in embryonic stem cells to generate a mouse line functionally lacking this subunit (16). Exons coding for the CNGA4 pore region, two transmembrane domains, and the cyclic nucleotide-binding region (CNb) were deleted, as were three intervening introns (Fig. 1A), ensuring that no functional protein could be expressed (17). In situ hybridization experiments verified the presence of CNGA4 mRNA in ORNs of wild-type (+/+) and heterozygous (+/−) animals but not in null (−/−) mice (16) (Fig. 1B), demonstrating that the targeted deletion of the CNGA4 gene abolished CNGA4 subunit expression.

Development of a normal olfactory system requires CNGA2-dependent neural activity (18–20). Because CNGA4 may itself form an ion channel insensitive to cyclic nucleotides but sensitive to other second messengers (21), we investigated whether CNGA4 might also be required for normal development of the olfactory epithelium and olfactory bulb. In situ hybridization studies showed that the olfactory marker protein mRNA (16) and protein (22), a signature of mature ORNs, was maintained in −/− mice, indicating that ORN development was not disrupted (22). The mRNA for major components of the transduction machinery was also normal, including CNGA2, CNGB1b, the heterotrimeric GTP-binding protein subunit Go, and the adenylyl cyclase ACIII (16, 22). Tyrosine hydroxylase expression in the periglomerular cells of the olfactory bulb, a correlate of afferent activity (23), remained high in both CNGA4−/− and wild-type animals (Fig. 1C), in contrast to expression in mice lacking CNGA2 (18). Therefore, we believe the CNGA4 subunit is not essential for normal development of the olfactory epithelium or bulb.

Heterologous expression studies have shown that homomeric channels formed by CNGA2

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**Fig. 1.** Targeted disruption of the CNGA4 gene abolishes CNGA4 expression and shifts the cyclic nucleotide sensitivity of the native CNG channel. (A) Exon-intron structure of the targeted region of the CNGA4 gene (top), restriction map of the wild-type locus (middle), and targeting vector (bottom). S2, S3, and S4, transmembrane domains 2, 3, and 4; P, channel pore; CNb, cyclic nucleotide-binding domain; d3, Hind III; Bs, Bsi EI; Sa, Sac I; Bg, Bgl II; Xh, Xho I. The proper gene targeting of this locus was confirmed by Southern blot hybridization (22). (B) In situ hybridization with CNGA4 probe on OE from CNGA4 +/+; +/−, and −/− mice. (C) Tyrosine hydroxylase immunohistochemistry (16) on sections of olfactory bulb from CNGA4 +/+ and −/− mice. Signal is observed in the glomerular layer (GL) and absent from the outer nerve layer (ONL) and the external plexiform layer (EPL). (D) Currents induced by 1 mM and 10 μM cAMP, respectively, from an excised, inside-out membrane patch from the dendritic knob of a +/+ (left) and a −/− (right) ORN. Buffered zero-Ca2+ Mg2+ Tyrode’s solution was present on both sides of membrane. In each trace, the current is activated by a 90-ms voltage step from 0 to −60 mV. Data represent the average of 10 sequential voltage steps. (E) The cAMP dose response relationships after averaging and normalization for channels from +/+; +/−, and −/− mice. +/+: one mouse, three patches; +/−: two mice, four patches; −/−: two mice, six patches. Error bars represent SEM. Smooth curves are fits by the Hill equation, with K1/2 values and Hill coefficients of 4.9 μM, 1.91 (+/+); 4.5 μM, 1.66 (+/−); and 55.2 μM, 1.10 (−/−), respectively. (F) EOG responses measured from the ciliary layer of a +/+ and a −/− OE. Stimulation was a single 500-ms pulse of IBMX at the indicated concentrations (in μM) ejected from a multibarrel pipette. Maximum responses from the two kinds of animals were not significantly different (mean ± SD = 655 ± 165 μV for +/+ and 593 ± 267 μV for −/−; more than six measurements were taken from four or five mice in each case). (G) Averaged and normalized IBMX dose-response relations from +/+ (solid squares), +/− (open squares, dashed blue line), and −/− (solid circles, red line) mice. Error bars represent SEM. Smooth curves are fit by the Hill equation, with K1/2 value and Hill coefficient of 28.3 μM, 1.9 (+/+); 27.2 μM, 1.7 (+/−); and 82.4 μM, 2.0 (−/−), respectively.
differ from the native olfactory channel with regard to several functional characteristics, including a much higher concentration of cAMP required for half-maximal ($K_{1/2}$) of the channel (12–15). The coexpression of CNGA2 with CNGA4 or CNGB1b, however, results in a cAMP $K_{1/2}$ value closer to that of the native channel, whereas the presence of all three subunits in the heterologous system further shifted the channel sensitivity to the native value. To assess the function of CNGA4 in the native channel, cAMP-activated currents were recorded in inside-out membrane patches excised from dendritic knobs of +/-, +/-, and -/- ORNs (16). Olfactory channels from the -/- animals exhibited a decreased affinity for cAMP, with a dose-response relation shifted by about 10-fold to higher cAMP concentrations; the behavior of the channels from +/- ORNs was like that of wild-type channels (Fig. 1, D and E). To determine whether this shift in channel sensitivity occurred as a change in the cell response, electro-olfactograms (EOGs) were recorded from +/-, +/-, and -/- mice (16) in response to a 500-ms pulse of isobutyl methyl xanthine (IBMX), which elevates intracellular cAMP by inhibiting endogenous phosphodiesterase activity (Fig. 1, F and G). The IBMX dose-response relations (24) for +/- and +/- mice were essentially identical, but for -/- mice it was shifted by about threefold, to a higher IBMX concentration (Fig. 1G). The shift in the dose-response relation for -/- mice indicates that CNGA4 contributes to the high cAMP sensitivity of the native olfactory channel.

ORNs from CNGA4 null mice displayed an unanticipated defect in odor adaptation. Odor adaptation, a decrease in sensitivity arising from prolonged or prior odor exposure, was examined by eliciting an EOG from the olfactory epithelium. With an 8-s odor stimulus (cineole, 100 μM), adaptation in +/- mice was evident from the progressive reduction of the EOG response during the stimulus (Fig. 2A). The response phenotype in the CNGA4 +/- mice was different, in that the desensitization rate was reduced by about eightfold (Fig. 2, B and C). Application of IBMX at several subsaturating concentrations also caused a significant decrease in the desensitization rate (Fig. 2, B and C). This drastic difference in the speed of adaptation between wild-type and mutant mice was also observed in a paired-pulse paradigm (8, 25). For CNGA4 +/- mice exposed to cineole (100 μM, 500 ms), the peak amplitude of the EOG response to the second stimulus (after a 3-s interstimulus interval) was only half that of the first (Fig. 2D). For CNGA4 -/- mice, on the other hand, the first and second responses were nearly identical. The same results were obtained with IBMX as a stimulus (Fig. 2, E and F). Hence, the presence of CNGA4 in the native olfactory channel accelerates the adaptation of ORNs to odor stimulation.

The similarly altered adaptation kinetics between wild-type and CNGA4 +/- animals, regardless of whether odorant or IBMX was used as a stimulus, supports the notion that the underlying mechanism resides at the channel level, most likely in the form of Ca2+-mediated inhibition through calmodulin. Because the permeability of the heterologously expressed channel to Ca2+ is not significantly altered in the absence of CNGA4 (26), we assessed whether CNGA4 is necessary for the rapid binding of CNGA4-calmodulin to the channel complex. Exposure of excised membrane patches to 50 μM Ca2+ and 250 nM CaM produced broadly similar shifts in the dose-response relation between current activation and cAMP concentration for wild-type (4.3-fold) and CNGA4 (-3.0-fold) channels (Fig. 3A). This indicated that the steady-state modulation of the native olfactory channel by Ca2+-CaM does not require CNGA4, and this is consistent with previous work using heterologous expressions of the channel subunits (6, 7, 27). However, a large difference in the onset kinetics of the Ca2+-CaM modulation was observed. In excised-membrane-patch experiments (26), application of 50 μM Ca2+ and 1 μM CaM produced a rapid decrease in the CNGA4-activated current for wild-type (Fig. 3B) and CNGA4 +/- channels (22). For CNGA4 +/- channels, however, the decrease in current by the same concentrations of Ca2+ and CaM was slowed by almost 70-fold (Fig. 3, C and D). The recovery of the current after removal of Ca2+ and CaM was several-fold faster for -/- than for +/- channels (+/- channels were like those of wild-type animals) (Fig. 3E), suggesting that the presence of CNGA4 also reduced to some degree the disinhibition rate when Ca2+ levels fell. The decelerated Ca2+-CaM–induced inhibition and the subsequent faster recovery may explain the adaptation defect manifested by the EOG of CNGA4 +/- animals in the long-pulse as well as the paired-pulse experiments; namely, the cAMP affinity of the CNGA4 +/- channel is much more resistant to change by the presence of Ca2+-CaM during odorant stimulation. In these experiments, we cannot resolve whether CNGA4 alters the kinetics of Ca2+-CaM binding to the channel, the gating kinetics of the Ca2+-CaM–bound channel, or both. In heterologous expression where CNGA4 is expressed, Ca2+-CaM binds to the channel equally well in the open or closed state; in the absence of CNGA4, Ca2+-CaM preferably binds to the channel in the closed state (28). Our results on the native channel are consistent with this observation.

Our experiments using gene deletion dem-
onstrate the importance of Ca\(^{2+}\)-CaM-dependent channel modulation in the adaptation of native olfactory neurons to odors, indicating the critical role of the CNGA4 subunit in accelerating this adaptation. Thus far, among native CNG channels, only the olfactory channel is known to have three, instead of two, distinct subunits, including CNGA4. In correlation with this unique feature, the olfactory channel is also the only native CNG channel in which direct Ca\(^{2+}\)-CaM modulation serves as a major negative feedback mechanism in signal transduction [unless the situation in retinal photoreceptors (29)]. Rapid olfactory adaptation allows an animal to continually assess changes in odor environment and intensity that are essential to follow odor plumes and trails. The presence of CNGA4 in the olfactory channel contributes to this feat.

References and Notes

1. Different nomenclature has been under consideration in the field, and we and the Bradley group (28) have used a common nomenclature based on an informal survey of 20 colleagues in the field. Our discussion and proposal are described in a letter in this issue (www.sciencemag.org/cgi/content/summary/294/5549/2093a).


16. For supplemental data on the expression of transduction components, and for detailed methods, see Science Online at www.sciencemag.org/cgi/content/full/294/5549/2172/DC1.

17. β-galactosidase histochemistry of olfactory epithelium and the olfactory bulb revealed a faint but consistent expression pattern that only partially mimicked the native CNGA4 expression pattern. One explanation for this observation is that critical enhancer sequences were eliminated by the deletion and that reporter expression reflected the influence of nearby regulatory elements. The known presence of olfactory receptor genes in proximity to the CNGA4 gene might account for this zonal-restricted, scattered expression pattern observed in the olfactory epithelium (30).


22. S. D. Munger et al., data not shown.


24. IBMX was used in these experiments because it induced responses in all olfactory cilia; useful dose-response relationships could not be determined for odors because saturation was never effectively achieved. These EOG measurements cannot be compared quantitatively with the excited-patch experiments because the EOG represents the contributions of both the CNG and a Ca\(^{2+}\)-gated Cl\(^{-}\) conductance in these cells.


27. For unclear reasons, the shift in the CaM dose-response relation observed here due to Ca\(^{2+}\)-CaM was smaller than previously reported [6].


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