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Functional Analysis Of The Guanylyl Cyclase Type D Signaling System In The Olfactory Epithelium

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Abstract

The mammalian olfactory system recognizes a wide range of chemical stimuli. The majority of cells in the main olfactory epithelium (MOE) use a cAMP-mediated signaling system to transduce odor signals. However, a subset of MOE neurons instead expresses components of a cGMP signaling cascade, including the receptor guanylyl cyclase GC-D and the cyclic nucleotide-gated channel subunit CNGA3. We used a combination of molecular biological, physiological and imaging approaches to characterize this neuronal population. Neurons expressing GC-D show excitatory responses to the natriuretic peptide hormones uroguanylin and guanylin, as well as to stimuli present in urine, that are dependent on both GC-D and CNGA3. Though all GC-D-expressing neurons are highly sensitive to these stimuli, individual cells are differentially tuned to either one or both of the peptides. Together, these findings suggest that neurons expressing GC-D are part of a specialized olfactory subsystem that is responsive to semiochemicals.

Keywords

semiochemical; cGMP; necklace glomeruli; natriuretic peptides; GC-D; PDE2

A mammal's ability to detect, discriminate, and respond to a wide variety of chemosensory cues is critical for its survival. For example, chemosensory stimuli signal the sources and quality of food, the sexual status of other individuals, or the presence of predators. Within the main olfactory system, canonical olfactory sensory neurons (OSNs) detect and transduce odors through a receptor-mediated, G protein-coupled signal transduction mechanism that results in an increase in intracellular cAMP, depolarization of the neuron, and the generation of action potentials sent to the olfactory forebrain.^{1, 2} However, a small subset of OSNs in the MOE lacks the cAMP-mediated signal transduction system found in canonical OSNs. Instead, these cells specifically express a member of the receptor guanylyl cyclase family, GC-D,³ as well as the cGMP sensitive phosphodiesterase PDE2⁴ and a cGMP-specific cyclic nucleotide gated channel subunit, CNGA3.⁵ GC-D-expressing (GC-D⁺) OSNs project their axons to a group of glomeruli encircling the caudal main olfactory bulb (MOB), the necklace glomeruli.⁴ However, the function of this putative olfactory subsystem, the GC-D⁺ neurons and their glomerular targets, remained unclear. Recently, we combined gene-targeting in mice with immunohistochemical, electrophysiological, and optical imaging approaches to functionally characterize this novel subset of sensory neurons.⁶

Here, we summarize many of our key findings and discuss their implications for olfactory function.

We reasoned that responses to stimuli for GC-D⁺ neurons would be present in mice lacking an intact canonical olfactory transduction pathway, but absent in mice lacking GC-D. We recorded electroolfactogram (EOG) responses from *Cnga2* null mice (which lack an intact cAMP-mediated olfactory transduction cascade and do not respond to most odors⁷) to a number of compounds found in urine, a rich source of pheromones and other semiochemicals in mice.⁸ We observed robust responses to two natriuretic peptide hormones, uroguanylin and guanylin, but not to the GC-A ligand urodilatin or to heat stable enterotoxin, a GC-C ligand.⁶ We next examined mice in which the gene encoding GC-D, *Gucy2d*, had been deleted and replaced with a reporter cassette consisting of an internal ribosomal entry site (IRES) followed by a gene encoding a tau-β-galactosidase fusion protein (*Gucy2d*^{-/-} mice). Though uroguanylin and guanylin responses were present in MOE of *Gucy2d*^{+/-} and *Gucy2d*^{+/+} mice, EOGs recorded from *Gucy2d*^{-/-} mice showed no response to the peptides. A similar loss of uroguanylin and guanylin responses was seen in *Cnga3*^{-/-} mice. Together, these results indicate that GC-D⁺ neurons are required for uroguanylin and guanylin responses in the MOE.

Odor stimulation of canonical OSNs results in an elevation of intracellular cAMP followed by membrane depolarization and action potential firing.^{1, 2} In contrast, light stimulation of mammalian photoreceptors leads to a decrease of cGMP and hyperpolarization of these cells.⁹ To determine if chemosensory responses of GC-D⁺ neurons are excitatory or inhibitory, we recorded action potentials using cell attached patch clamp recording from β-galactosidase⁺ (i.e., GC-D⁺) OSN dendritic knobs in *Gucy2d*^{-/-} and *Gucy2d*^{+/-} mice.⁶ β-galactosidase⁺ neurons in *Gucy2d*^{+/-} mice, which were visualized using a fluorescent β-galactosidase substrate, responded to stimulation with uroguanylin, guanylin, dilute urine, or the control stimulus, KCl, with an increase in action potential firing. In contrast, β-galactosidase⁺ neurons from *Gucy2d*^{-/-} mice responded only to the KCl control. Both groups of cells responded to stimulation with the cGMP analog 8-bromo-cGMP. Thus, chemosensory responses in GC-D⁺ neurons are excitatory and dependent on GC-D. These results were confirmed by immunohistochemical analyses of tyrosine hydroxylase (TH) expression in the necklace glomeruli of *Gucy2d*^{+/-}, *Gucy2d*^{-/-} and *Cnga3*^{-/-} mice.⁶ TH expression in periglomerular cells of the MOB, which is a correlate of afferent activity to glomeruli,^{10, 11} was specifically reduced in necklace glomeruli of *Gucy2d*^{-/-} and *Cnga3*^{-/-} mice, but maintained in the necklace glomeruli of *Gucy2d*^{+/-} mice. Therefore, both GC-D and *Cnga3* are necessary to maintain normal afferent activity to necklace glomeruli.

To understand the diversity of responses across the GC-D⁺ population, we imaged dendritic knobs of β-galactosidase⁺ OSNs, again visualized with a fluorescent β-galactosidase substrate, for stimulus-induced changes in intracellular Ca²⁺.⁶ All β-galactosidase⁺ knobs in the MOE of *Gucy2d*^{+/-} mice, but not *Gucy2d*^{-/-} mice, exhibited increases in intracellular Ca²⁺ upon stimulation with urine, a mixture of uroguanylin and guanylin, or with either of the phosphodiesterase inhibitors IBMX and EHNA. The observation that a mixture of only two peptide stimuli is capable of activating virtually all GC-D⁺ neurons is in stark contrast to stimulus coding by canonical OSNs, where single odorants activate only a small subset of OSNs.^{1, 2} However, individual peptides stimulated only subsets of β-galactosidase⁺ knobs, indicating that GC-D⁺ neurons can be classified into three functional populations: those that respond to uroguanylin but not guanylin, those that respond to guanylin but not uroguanylin, and those that respond to both peptides. Sensitivity to uroguanylin and guanylin was restricted to GC-D⁺ neurons, since no responses to these peptide stimuli were observed in β-galactosidase-negative knobs.

GC-D⁺ OSNs are highly sensitive chemosensory detectors of the natriuretic peptide hormones uroguanylin and guanylin. In the gut and kidney, these peptides act to regulate sodium and fluid balance through their stimulation of the receptor guanylyl cyclase GC-C. ¹² It is intriguing to speculate that uroguanylin (which is present in significant quantities in urine) and guanylin (which may be present under some conditions) could communicate information about metabolic state, thirst or satiety between individuals. At any rate, our multifaceted analysis of GC-D and the MOE neurons that express it identifies guanylin and uroguanylin as novel mammalian semiochemicals that are recognized by a unique olfactory detection system. The identification and characterization of this distinct OSN population sheds new light on the complexity of chemical communication in mammals.

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Figure.

(A) X-gal histochemical labeling of the olfactory epithelium (left) and olfactory bulb (right; lateral view) of a *Gucy2d* $-/-$ mouse reveals axons of GC-D+ neurons (blue) entering necklace glomeruli (arrows indicate two of the necklace glomeruli). The location of the cribriform plate is indicated by the dashed line. (B) Dose dependency of peak responses to uroguanylin (UG) and guanylin (G) obtained from *Gucy2d* $+/+$, $+/-$, or $-/-$ mice. Data points are normalized to the mean response of a given stimulus at $1 \mu\text{M}$ in *Gucy2d* $+/+$ mice. Insets: examples of the time course of peptide-induced responses in *Gucy2d* $+/+$ or $-/-$ mice (ligands $1 \mu\text{M}$ each). Scale bars: $40 \mu\text{V}$, 1 s . (C) Patch clamp recording from individual dendritic knobs of GC-D cells. Stimulus-evoked discharges recorded from β -gal-positive knobs of *Gucy2d* $+/-$ (left) or $-/-$ (right) mice. KCl (60 mM); uroguanylin (UG; $1 \mu\text{M}$); guanylin (G, $1 \mu\text{M}$); dilute urine ($1:100$). Arrows: stimulus application. (D) Example of Ca^{2+} knob responses, assayed by confocal imaging of Ca^{2+} fluorescence, to UG ($1 \mu\text{M}$), G ($1 \mu\text{M}$), UG/G mixture ($1 \mu\text{M}$ each), and urine ($1:100$). No responses were observed in GC-D cells from *Gucy2d* $-/-$ mice. (E) Venn diagram indicating the proportions of GC-D+ neurons ($n=27$) responding to UG only, to G only, or to both peptides. Panels B-D reprinted, and panels A and E modified, with permission from ⁶; copyright 2007, National Academy of Sciences.