Ligand-Gated Chloride Channels Are Receptors for Biogenic Amines in *C. elegans*

The MIT Faculty has made this article openly available. Please share how this access benefits you. Your story matters.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>As Published</td>
<td><a href="http://dx.doi.org/10.1126/science.1169243">http://dx.doi.org/10.1126/science.1169243</a></td>
</tr>
<tr>
<td>Publisher</td>
<td>American Association for the Advancement of Science (AAAS)</td>
</tr>
<tr>
<td>Version</td>
<td>Author’s final manuscript</td>
</tr>
<tr>
<td>Accessed</td>
<td>Tue Dec 18 11:31:25 EST 2018</td>
</tr>
<tr>
<td>Citable Link</td>
<td><a href="http://hdl.handle.net/1721.1/84506">http://hdl.handle.net/1721.1/84506</a></td>
</tr>
<tr>
<td>Terms of Use</td>
<td>Creative Commons Attribution-Noncommercial-Share Alike 3.0</td>
</tr>
<tr>
<td>Detailed Terms</td>
<td><a href="http://creativecommons.org/licenses/by-nc-sa/3.0/">http://creativecommons.org/licenses/by-nc-sa/3.0/</a></td>
</tr>
</tbody>
</table>
Ligand-gated chloride channels are receptors for biogenic amines in *C. elegans*

Niels Ringstad¹,², Namiko Abe¹,²,³, and H. Robert Horvitz¹

¹HHMI, Department of Biology and McGovern Institute for Brain Research, MIT, Cambridge MA 02139

Abstract

Biogenic amines such as serotonin and dopamine are intercellular signaling molecules that function widely as neurotransmitters and neuromodulators. We have identified in the nematode *Caenorhabditis elegans* three ligand-gated chloride channels that are receptors for biogenic amines: LGC-53 is a high-affinity dopamine receptor, LGC-55 is a high-affinity tyramine receptor, and LGC-40 is a low-affinity serotonin receptor that is also gated by choline and acetylcholine. *lgc-55* mutants are defective in a behavior that requires endogenous tyramine, indicating that this ionotropic tyramine receptor functions in tyramine signaling *in vivo*. Our studies suggest that direct activation of membrane chloride conductances is a general mechanism of action for biogenic amines in the modulation of *C. elegans* behavior.

Biogenic amines function in diverse neuronal circuits as neurotransmitters and neuromodulators. Therapeutics for many psychiatric disorders, including major depression, schizophrenia and bipolar affective disorder, target signaling pathways of such biogenic amines as serotonin, dopamine, and noradrenaline (1). Biogenic amine signaling pathways are also targets of drugs of abuse (1). Almost all known biogenic amine receptors are *G* protein-coupled receptors (*GPCRs*) that signal through the activation of heterotrimeric guanine nucleotide-binding proteins (*G* proteins), which activate second-messenger signaling pathways. However, there exists a second type of biogenic amine receptor: biogenic amine-gated ion channels. The vertebrate 5-HT3 receptor is a serotonin-gated cation channel (2,3). Two arthropod histamine receptors and the *C. elegans* MOD-1 serotonin receptor are biogenic amine-gated chloride channels (4,5,6,7,8). These biogenic amine-gated channels from diverse phyla suggest a mechanism of action for their cognate ligands: fast excitation or inhibition analogous to the response to the activation of nicotinic acetylcholine receptors or to the activation of *GABA_A* receptors respectively. We report that such a mechanism of action for biogenic amines is more general: a single species, *C. elegans*, expresses multiple ion channels gated by biogenic amines.

The known biogenic amine-gated ion channels are members of the Cys-loop family of ion channels. Using database searches of proteins encoded by the *C. elegans* genome, we identified 26 presumptive Cys-loop family ion channels that are highly similar to the MOD-1 serotonin-gated chloride channel (*E* values reported by the BLAST algorithm range from 10⁻³⁵ to 10⁻⁹⁴, Table S1); 23 have sequences in their pore-forming M2 transmembrane domains predicted to confer chloride selectivity (9). We expressed these 26 receptors individually in *Xenopus laevis* oocytes and tested them for receptor activity using a two-electrode voltage clamp to monitor whole-cell currents evoked by application of a panel of agonists, including the biogenic amines serotonin, dopamine, octopamine, tyramine and histamine (10). We
identified three genes that encode ion channels activated by biogenic amines: lgc-40, lgc-53 and lgc-55. LGC-53 was activated with the highest efficacy by dopamine (Fig. 1A) with an EC50 of 4.4 μM (Fig. 1B). LGC-55 was activated with the highest efficacy by tyramine with an EC50 of 6.0 μM (Fig. 1C and D). LGC-40 was activated only by high concentrations of serotonin (EC50 = 905 μM) (Fig. 1E and F). We tested whether other potential ligands could be more effective and found that choline and acetylcholine gated LGC-40 at lower concentrations (choline EC50 = 3.4 μM, acetylcholine EC50 = 87 μM) (Fig. 1F) (11). Compounds that block ion channels endogenous to Xenopus oocytes failed to block agonist-evoked whole-cell currents in oocytes expressing LGC-40, LGC-53 and LGC-55, indicating that such endogenous channels did not mediate the agonist-evoked currents (Figure S1).

Ligand binding by Cys-loop family ion channels is mediated by the amino-terminal extracellular domains of channel subunits (12). We aligned the extracellular domain sequences of LGC-40, LGC-53 and LGC-55 with those of MOD-1 and 5-HT3 receptor subunits and of other Cys-loop family receptors but did not identify any amino acids that were conserved specifically among amine-gated channels (Figure S2).

All previously characterized dopamine receptors are GPCRs (1). We tested whether antagonists of G protein-coupled dopamine receptors can inhibit the LGC-53 dopamine-gated ion channel. Of seven antagonists tested (Fig. 2A), three acted with an IC50 of less than 100 μM: risperidone (IC50 = 40 μM), haloperidol (IC50 = 32 μM) and spiperone (IC50 = 38 μM) (Fig. 2B-D) (10). Risperidone, haloperidol and spiperone all are in clinical use as antipsychotics (1). Since these drugs also block LGC-53, we suggest that some of their actions as therapeutics might be through inhibition of a yet-to-be-identified human dopamine receptor that, like LGC-53, is composed of Cys-loop family channel subunits.

We tested d-tubocurarine and atropine, antagonists of ionotropic and G protein-coupled acetylcholine receptors, respectively (1), and hemicholinium-3, a blocker of the high-affinity choline transporter (13), for effects on choline-evoked currents in oocytes expressing LGC-40 (Fig. 2F-G). LGC-40 currents were blocked by low concentrations of d-tubocurarine (IC50 = 8.3 μM) but were relatively insensitive to atropine (Fig. 2F). Hemicholinium-3 was the most effective antagonist of the LGC-40 channel, with an IC50 of 2.0 μM (Fig. 2G). Thus, LGC-40 is sensitive both to a canonical antagonist of acetylcholine-gated ion channels as well as to an inhibitor of the high-affinity choline transporter.

The presumptive M2 regions, which are predicted to determine the ion selectivity of Cys-loop family ion channels, of LGC-40, LGC-53 and LGC-55 are more similar to the M2 regions of known chloride channels than to those of known cation channels (Fig. 3A) (9,12). To test whether these receptors are chloride channels, we measured the reversal potentials of agonist-evoked currents in Xenopus oocytes expressing LGC-40, LGC-53 and LGC-55 (Fig. 3B through D). In solutions containing 96 mM Na+ and 104 mM Cl−, ligand-evoked currents reversed at -23 to -26 mV, close to the reversal potential for chloride in Xenopus oocytes (14). Replacement of sodium ions with N-methyl D-glucuronate (NMDG) or choline did not alter the reversal potentials of the evoked currents. Replacement of chloride ions with gluconate shifted the reversal potentials positively by 17 to 31 mV (Fig. 3B through D) (15). These data indicate that LGC-40, LGC-53 and LGC-55 are chloride channels. The observed shift in reversal potential was less than predicted for channels that are perfectly selective for chloride over other anions (our unpublished observations); perhaps these channels might also pass the anions we used to replace chloride.

To identify biological functions of these amine-gated chloride channels, we isolated lgc-40, lgc-53 and lgc-55 deletion mutants (Figure S3). Lgc-55 mutants, which lack the tyramine-gated chloride channel, had a behavioral defect that indicated an in vivo function for this receptor in...
tyraminergic signaling. Tyramine from the RIM motor neurons suppresses small rapid head movements during touch-evoked locomotory reversals (16). Animals lacking endogenous tyramine fail to suppress these head oscillations and exhibit the Sho phenotype (Suppression of head oscillations-defective), as do animals lacking the tyraminergic RIM motor neurons (16) (Figure 4A). Similarly, *lgc-55* mutants continued to execute head movements during touch-evoked reversals (Fig. 4A). The Sho phenotype of *lgc-55* mutants was rescued by transgenes containing the *lgc-55* cDNA fused to *lgc-55* promoter sequences and by transgenes containing the *lgc-55* cDNA fused to the *unc-119* promoter, which drives expression broadly in the nervous system (17). To identify cells that express LGC-55, we created transgenic animals expressing GFP under the control of the *lgc-55* promoter (17). We observed expression of this reporter transgene in head muscles and in the glia-like GLR cells, which are connected to the head muscles by gap junctions (18), and weaker expression in many unidentified head neurons (Figure 4B). We also observed strong expression in the ALM and AVM mechanosensory neurons (data not shown). Expression of *lgc-55* in muscles, including in head muscles, did not rescue the Sho phenotype of *lgc-55* mutants (Fig. 4a). Our findings suggest that LGC-55 can act in the GLR cells or neurons to control head movements and establish that the LGC-55 tyramine receptor functions in vivo in the tyraminergic control of head movements.

No behavioral abnormalities were detected in mutants that carry deletions in *lgc-40* and *lgc-53* (19). We conclude that including MOD-1 there exist in *C. elegans* at least four ligand-gated chloride channels that can be activated by biogenic amines. Our results indicate that ligand-gated chloride channels constitute a class of receptor for biogenic amines and that direct activation of membrane chloride conductances is a general mechanism of biogenic amine action in *C. elegans*. Biogenic amine-gated chloride channels might exist in the nervous systems of animals other than *C. elegans* and arthropods. In molluscs, synaptic dopamine rapidly activates a chloride conductance that is blocked by picrotoxin, an antagonist of ligand-gated chloride channels (20,21,22). In the mammalian brain, histaminergic neurons project to the hypothalamus and use rapid inhibitory synaptic signals that are (i) not mediated by G protein signaling, (ii) are picrotoxin-sensitive and (iii) are mediated by chloride conductances (23). The molecular identity of the histamine receptor in this synapse is not known, but the recent demonstration that GABA<sub>A</sub> receptor subunits can form histamine-gated chloride channels in vitro suggests that an ionotropic histamine receptor might contain known ligand-gated chloride channel subunits (24). If direct activation of ligand-gated chloride channels is a mechanism of biogenic amine action in the mammalian brain, such receptors might be therapeutic targets, given the many links between aminergic signaling and psychiatric disorders (1,25).

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

### Acknowledgments

We thank Y. Kohara for *lgc-40, lgc-53* and *lgc-55* cDNA clones, A. Fire for expression vectors, R. O’Hagan for help with electrophysiology, A. Hellman for deletion screening, M. Alkema for discussions, J. Kehoe for bringing to our attention reports of dopamine-activated chloride conductances in molluscs, and D. Denning and B. Galvin for suggestions concerning the manuscript. This work was supported by NIH grant GM24663. N.R. received support from the Howard Hughes Medical Institute, the Life Sciences Research Foundation and The Medical Foundation. H.R.H. is an Investigator of the Howard Hughes Medical Institute.

### REFERENCES AND NOTES

10. Descriptions of how we expressed lgc-40, lgc-53 and lgc-55 in Xenopus laevis oocytes are in Supplementary Methods. We recorded whole-cell currents using a two-electrode voltage clamp (Warner Instruments). The ground electrode was connected to the recording chamber (Warner Instruments model RC-3Z) using an agar bridge. Data were acquired with Clampex 8.0 (Molecular Devices) and analyzed offline with Clampfit (Molecular Devices). Oocyte membrane potential was held at -60 mV for all experiments. To identify new Cys-loop family biogenic amine receptors, oocytes expressing candidate channels were sequentially superfused with ND96 (96 mM NaCl, 2.5 mM KCl, 1 mM MgCl₂, 1.8 mM CaCl₂, 5 mM HEPES, pH 7.6) containing GABA (100 μM), glycine (100 μM), glutamate (100 μM), histamine (100 μM), serotonin (1 mM), dopamine (100 μM), octopamine (100 μM) and tyramine (100 μM). Candidate receptors not activated by these ligands were also tested with ivermectin (10 μM), which can activate many ligand-gated chloride channels [Shan Q, Hadrill JL, Lynch JW. J Biol Chem 2001;276:12556. [PubMed: 11278873]].
11. The following compounds were tested for LGC-40-agonist activity: 5-acetyl 5-hydroxytryptamine, adenosine, adenosine triphosphate, carnosine, epinephrine, FLRFamide, FMRFamide, 5-hydroxytryptophan, homocarnosine, melatonin, methoxytyramine, norepinephrine, o-methyl 5-hydroxytryptamine, o-methoxylphenylethylamine, phenylethylamine, tryptamine. All chemicals were purchased from Sigma and tested at 1 mM concentrations, except for the FLRFamide and FMRFamide peptides, which were tested at 10 μM.
15. For ion replacement experiments, oocytes expressing LGC-40, LGC-53 or LGC-55 were held at a resting potential of -90 mV. LGC-40, LGC-53 or LGC-55 currents were evoked with 2 μM choline, 5 μM dopamine and 5 μM tyramine, respectively, in ND96 medium, sodium-free medium (96 mM cholineCl or NMDG-Cl replacing NaCl) or low-chloride medium (96 mM NaGluconate replacing NaCl). After 5 seconds, a voltage ramp of 20 mV/s from -90 mV to +60 mV was applied. Currents from voltage ramps recorded in the absence of ligands were subtracted from these current traces.
17. Descriptions of lgc-55 rescuing transgenes and the lgc-55 reporter transgene are in Supplementary Methods.
19. lgc-40 and lgc-53 mutants were grossly normal in locomotion, egg laying, defecation and pharyngeal pumping. We also tested lgc-53 mutants for defects in slowing in response to detection of a bacterial food source and for resistance to exogenous dopamine in locomotion and egg-laying assays. Supplementary Methods contains experimental details.

*Science. Author manuscript; available in PMC 2010 October 25.*
Figure 1. Biogenic amines evoke currents in *Xenopus* oocytes expressing LGC-53, LGC-55 or LGC-40

A, C, E. Whole-cell currents recorded from *Xenopus* oocytes expressing LGC-53, LGC-55 or LGC-40 during application of serotonin (5-HT), dopamine (DA), octopamine (OA), tyramine (TA) and histamine (HA). The concentrations of applied neurotransmitters were 10 μM in panels (A) and (B) and 100 μM in (C).

B, D. Dose-response curves for dopamine and tyramine-evoked currents in *Xenopus* oocytes expressing LGC-53 and LGC-55, respectively. Each point (± s.e.m.) represents the average of three to five recordings. Dose-response data were fitted to the Hill equation and normalized to $I_{\text{max}}$. The half-effector concentration (EC50) of dopamine for LGC-53 was 4.4 μM, and the estimated Hill coefficient was 1.9. The half-effector concentration of tyramine for LGC-55 was 6.0 μM, and the estimated Hill coefficient was 1.8.

F. Dose-response curves for serotonin, acetylcholine and choline-evoked currents in *Xenopus* oocytes expressing LGC-40. Dose-response data were fitted to the Hill equation and normalized to $I_{\text{max}}$. $n \geq 5$. The half-effector concentration of serotonin for LGC-40 was 905 μM, and the estimated Hill coefficient was 2.7. The half-effector concentrations of acetylcholine and choline for LGC-40 were 87 μM and 3.4 μM, respectively, and the estimated Hill coefficients were 1.5 and 1.9, respectively.
Figure 2. Pharmacological characterizations of the ionotropic dopamine receptor LGC-53 and the ionotropic choline receptor LGC-40

A. Inhibition of LGC-53 currents by dopamine-receptor antagonists. The mean ratios (± s.e.m.) of the peak currents evoked in the presence and absence of the indicated dopamine receptor antagonist are shown. \( n \geq 5 \). Drugs were tested at a concentration of 100 \( \mu M \), and 5 \( \mu M \) dopamine was used to evoke LGC-53 currents.

B-D. Dose-response curves for the inhibition by haloperidol, risperidone, and spiperone of LGC-53 currents. 5 \( \mu M \) dopamine was used to evoke currents in oocytes expressing LGC-53 in the presence of different concentrations of dopamine receptor antagonists. Currents were normalized to the current evoked by 5 \( \mu M \) dopamine in the absence of receptor antagonists. \( n \geq 5 \).

E-F. Dose-response curves for the inhibition by atropine, d-tubocurarine, and hemicholinium-3 of LGC-40 currents. Currents were evoked by 2 \( \mu M \) choline in oocytes expressing LGC-40 in the presence of different concentrations of compounds. Currents were normalized the current evoked by 2 \( \mu M \) choline in the absence of any compounds. \( n \geq 5 \).
Figure 3. LGC-40, LGC-53 and LGC-55 are chloride channels
A. Alignment of sequences from the presumptive M2 region of amine-gated ion channels, which determines ion selectivity of Cys-loop family ion channels (9,12). Cation-selective 5-HT3 receptor subunits are labeled in green, and anion-selective MOD-1, HCLA, and HCLB subunits are labeled in red.
B-D. I-V curves of LGC-40, LGC-53 and LGC-55 in ND96 medium (which contains 96 mM sodium and 104 mM chloride), sodium-free medium (0 mM sodium and 104 mM chloride) or low-chloride medium (96 mM sodium and 8 mM chloride). The mean reversal potential ± s.e.m. from four to five experiments under each condition is shown.
Figure 4. LGC-55 is required for the tyraminergic modulation of head movements by *C. elegans* and is expressed in the GLR glia-like cells and head muscles

A. The fractions of animals that are suppression of head oscillations-defective (Sho) are plotted for the wild type, *tdc-1*, and *lgc-55* mutants and *lgc-55* mutants carrying rescuing transgenes that express the *lgc-55* cDNA using its own promoter, the pan-neuronal *unc-119* promoter, or the pan-muscle *myo-3* promoter. In each experiment we tested 20 individuals of each genotype for the Sho phenotype. The mean fraction of animals with the Sho phenotype ± s.e.m. is plotted, \( n \geq 3 \). Three independent lines (labeled #1-3) carrying each transgene were assayed.

B. Expression of *lgc-55*. An *lgc-55∷gfp* reporter transgene is expressed in the GLR glia-like cells and head muscles (16). Arrowheads indicate some of the unidentified head neurons that express the reporter transgene. The tyraminergic RIM neurons, which provide the tyramine that inhibits head movements during reversals, are labeled with a *tdc-1∷dsRed* reporter transgene.