A Family of Acetylcholine-gated Chloride Channel Subunits in Caenorhabditis elegans*

Received for publication, November 8, 2004, and in revised form, December 2, 2004
Published, JBC Papers in Press, December 3, 2004, DOI 10.1074/jbc.M412644200

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The genome of the nematode Caenorhabditis elegans encodes a surprisingly large and diverse superfamily of genes encoding Cys loop ligand-gated ion channels. Here we report the first cloning, expression, and pharmacological characterization of members of a family of anion-selective acetylcholine receptor subunits. Two subunits, ACC-1 and ACC-2, form homomeric channels for which acetylcholine and arecoline, but not nicotine, are efficient agonists. These channels are blocked by D-tubocurarine but not by α-bungarotoxin. We provide evidence that two additional subunits, ACC-3 and ACC-4, interact with ACC-1 and ACC-2. The acetylcholine-binding domain of these channels appears to have diverged substantially from the acetylcholine-binding domain of nicotinic receptors.

Fast (ionotropic) cholinergic neurotransmission is generally mediated by nicotinic acetylcholine (ACh)1 receptors (nAChRs). These are cation-selective channels and hence mediate excitatory neurotransmission. However, electrophysiological evidence of ionotropic, ACh-gated chloride channels in molluscs suggests the existence of fast inhibitory cholinergic neurotransmission as well (1–3). The ACh-gated chloride channels in Aplysia neurons respond to several agonists and antagonists of nAChRs, indicating that, like the nAChRs, they may belong to the superfamily of Cys loop ligand-gated ion channel (LGIC) subunits. Otherwise, little is known about the molecular nature of the receptors that mediate fast inhibitory cholinergic neurotransmission, whether this type of neurotransmission is widespread in the animal kingdom, or how it evolved.

The Cys loop LGICs are encoded by a large and diverse gene superfamily. These channels are pentameric and can be homomers or heteromers consisting of as many as four different subunits, each encoded by a different gene (4). Subunits of the Cys loop LGIC superfamily share a topology that consists of a large extracellular ligand-binding domain and four transmembrane domains that form the ion-selective pore (4–6). In vertebrates, the LGIC superfamily consists of two families of cation-selective channels, the nicotinic ACh receptors and the 5-hydroxytryptamine type 3 receptors, and two families of anion channels, the GABAa receptors and the glycine receptors (7). The repertoire of invertebrate LGICs is larger, including, in addition to homologues of vertebrate channels, histamine-gated chloride channels (8, 9), a GABA-gated catation channel (10), a serotonin-gated anion channel (11), several glutamate-gated anion channels (12–16), and a divergent choline-gated nAChR (17). Thus, the LGIC channel structure appears flexible enough to accommodate diverse ligands and ligand/ion selectivity pairings.

Although no genes encoding ACh-gated chloride channels have been previously identified, it is likely that many invertebrate receptors with unusual properties remain to be characterized. The genomes of Caenorhabditis elegans and Drosophila melanogaster reveal numerous predicted Cys loop LGICs that do not obviously belong to any family of known ion or ligand specificity (18–21). C. elegans in particular encodes ~70 LGIC subunit genes, of which fewer than 20 have been characterized pharmacologically (19). The function of such a large and diverse LGIC superfamily in a single species is unclear.

To better understand the constraints on Cys loop LGIC structure and evolution and to identify new modes of neurotransmission, we have characterized several members of a novel family of channel subunits from C. elegans. These form ACh-gated chloride channels exhibiting an unusual pharmacology that appears to reflect a unique ACh-binding site.

EXPERIMENTAL PROCEDURES

Cloning ACC cDNAs—Poly(A)1 RNA was purified from adult worms (Bristol N2 strain). First strand cDNA was synthesized with oligo(dT) primer using the avian myeloblastosis virus reverse transcriptase system (Invitrogen Canada Inc., Burlington, Ontario, Canada). The open reading frame of ACC-1, -2, and -4, as predicted in Wormbase (available on the World Wide Web at www.wormbase.org/), was amplified by PCR using the following primers: ACC-1, 5'-GGGGACACAAGTTTGTACAAAAAAGCAGGCTGGA- TTAT-3' and 5'-GGGGACACTTTGTGACAAAAAAGGCTCGTATGAGTCATCCGGGTTGGA- TTAAAGGTGATCAATATTCACA-3', ACC-2, 5'-GGGGACAGTTTGTACAAAAAAGCAGGCTGGA- TTAT-3' and 5'-GGGGACACCTTTGTGACAAAAAAGGCTCGTATGAGTCATCCGGGTTGGA- TTAAAGGTGATCAATATTCACA-3', ACC-3, 5'-GGGGACACATATGCGACTACATGATATTTACTCTTTTATCAACACTGCTCT-3' and 5'-GGGGACACTTTGTGACAAAAAAGGCTCGTATGAGTCATCCGGGTTGGA- TTAAAGGTGATCAATATTCACA-3', ACC-4, 5'-GGGGATCCATATGCGACTACATGATATTTACTCTTTTATCAACACTGCTCT-3' and 5'-GGGGACACTTTGTGACAAAAAAGGCTCGTATGAGTCATCCGGGTTGGA- TTAAAGGTGATCAATATTCACA-3'. PCR products were subcloned either into pDONR vector. This paper is available online at http://www.jbc.org
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gene were sequenced to determine the true open reading frames and to
find possible mutations resulting from reverse transcription-PCR. Non silenced mutations were fixed either by overlap extension PCR (22) or by splicing together mutation-free cDNA fragments using convenient restriction sites.

Sequence Analysis—Amino acid sequences were aligned using the ClustalW program (available on the World Wide Web at clustalw.genome.ad.jp). Transmembrane domains were predicted using the TMHMM method based on a hidden Markov model (23). SignalP 2.0, NetGlyc 1.0, and NetPhos 2.0 programs based on artificial neuronal networks were used to predict signal peptide sequences (24). All of the above mentioned prediction programs are available on the World Wide Web at www.cbs.dtu.dk/services.

Expression in Xenopus Oocytes and Electrophysiology—cDNAs were subcloned into the pITN expression vector (25). The pITNcDNA con structs were linearized with Sall (ACC-2) or BamHI (ACC-1, -3, and -4), and capped cRNAs were transcribed using the MEGAscript Kit (Ambion, Austin, TX). Synthesized cRNAs were recovered by LiCl precipitation and resuspended in nuclease-free H2O at a final concentration of 1 μg/ml.

Oocytes were harvested from mature female Xenopus laevis according to standard procedures (26). Oocytes were maintained at 20 °C in ND96 solution (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, and 5 mM Hepes, pH 7.5) supplemented with 100 μM gentamicin and 2 mg/ml pyruvate. Oocytes were injected with 40 nl of cRNA using the Microinjector (Narishige, Hamden, CT) or a Maltese Cross chamber (ALA Scientific Instruments, Westbury, NY). Data were acquired at 1 kHz using Clampex software (Axon Instruments, Foster City, CA). All drugs were obtained from Sigma. Dose-response curves for agonists were generated by applying increasing concentrations of drug followed by 3–9-min washes to determine the EC50 and Hill coefficient, dose-response curves (as smooth curves in graphs) were fitted using the Hill equation as follows,

\[
f(I) = \left( \frac{I_{\max}}{I_0 + [I]} \right) + I_{\min}
\]

where \(I\) represents the normalized response, \(I_{\max}\) is the maximal response elicited by a saturating concentration of agonist, \(I_0\) is the concentration of agonist inducing half-maximal response, \(n\) is the Hill coefficient, and \(I_{\min}\) is the normalized response at lowest agonist concentration.

Oocytes were preincubated with antagonist for 1 min (10 min for α-BT) prior to co-application of the antagonist with either 1 μM or 10 μM ACh for oocytes expressing ACC-1 or ACC-2, respectively. The amplitude of response to co-application of ACh and antagonist was normalized to that of the response to ACh alone and expressed as a percent of the response to ACh alone at 5 min.

The ACC-1 and ACC-2 putative subunits, such as a signal sequence, an N-terminal extracellular domain with the Cys loop, four transmembrane domains (M1–M4), and a large cytoplasmic loop located between M3 and M4, were recognizable in the amino acid sequences of these proteins (Fig. 1B). The ACC-1 and ACC-2 putative proteins are 40% identical to each other at the amino acid level and exhibit 31 and 29% identity to MOD-1, respectively. In contrast, ACC-2 shows 23% identity to UNC-49B, a C. elegans GABA-gated chloride channel subunit (27), and is only 16% identical to UNC-38, a C. elegans nAChR subunit.

ACC-1 and -2 Form ACh-gated Chloride Channels—To determine the ligand specificity of the ACC subunits, we expressed ACC cRNAs in Xenopus oocytes and voltage-clamped the oocytes at −80 mV. ACC-1- and ACC-2-injected oocytes exhibited an ACh-elicted inward current with maximal magnitude varying from 0.8 to 3.4 μA (Fig. 2, A and C). The ACC-1-dependent current showed almost no desensitization even at saturating ACh concentrations, whereas the ACC-2-dependent current desensitized. ACC-1 responded to ACh with a half-effector concentration (EC50) of 0.26 ± 0.01 μM and an estimated Hill coefficient of 1.26 ± 0.04 (Fig. 2B), whereas ACC-2 was much less sensitive, responding with an EC50 of 9.54 ± 0.11 μM and a Hill coefficient of 2.04 ± 0.08 (Fig. 2D).

Oocytes injected with ACC-3 cRNAs exhibited a weak response (10–30 nA) to 1 mM ACh, and those injected with ACC-4 showed no response. An ACh-induced current was not detected in distilled H2O-injected or noninjected oocytes. Oocytes expressing ACC cRNAs did not respond to 1 mM GABA, glutamate, glycine, histamine, or dopamine (not shown). ACC-2-injected oocytes responded slightly to 1 mM serotonin (<2% of the maximal ACh response) and to octopamine (<1%), but no response to these compounds was observed in oocytes expressing other ACC subunits.

ACC-1, -2, and -3 share a proline-alanine motif of the second transmembrane domain (corresponding to the intermediate ring of nAChRs) that has been shown to confer anion selectivity in vertebrate GABA and glycine receptors (28–30) (Fig. 1B). To determine whether ACC-1 and ACC-2 are also chloride channels, we generated I–V curves. The reversal potentials in ND96 for ACC-1 or ACC-2 homomeric channels were −18.7 ± 1.4 mV and −18 ± 1.6 mV, respectively, consistent with the equilibrium potential for chloride (Fig. 3, A and B) (31). When the nonpermeant anion gluconate was substituted for chloride (7.6 mM final external chloride), there was a positive shift in the reversal potential of 65.7 mV (ACC-1) and 68.6 mV (ACC-2). In contrast, when arginine was substituted for sodium, the shift in reversal potential was negligible (ER reversal = −17.2 ± 1.7 mV, ACC-1, and −17.0 ± 1.0, ACC-2) (Fig. 3, B and C). We observed shifts of 59.8 ± 4.9 and 54.4 ± 3.7 mV in the reversal potential for a 10-fold change in chloride concentration for ACC-1 and ACC-2, respectively (Fig. 3C). This is in agreement with the theoretical shift of 58 mV predicted by the Nernst equation for chloride-selective channels.

The Pharmacology of the ACC Channels Reflects a Unique Ligand-binding Site—The sequence identity between the ACCs and nAChRs in their extracellular ligand-binding domains is relatively low. More specifically, the six ligand-binding loops (A–F), as predicted from lineups with nAChRs, are not well conserved between nAChRs and the ACCs (Fig. 1B). Therefore, we predicted that the ACCs would have a unique pharmacological profile. We tested agonists and antagonists of vertebrate nAChRs on the ACC receptors. The classical nAChR agonist nicotine at 1 mM was a poor agonist of the ACC channels (Fig. 4A). At 0.5 mM, nicotine was a partial agonist of ACC-1 but
Fig. 1. The ACC family of LGICs. A, a neighbor joining tree of the LGIC subunit superfamily polypeptides in C. elegans (ce) with acetylcholine-binding protein (AChBP), selected rat (v), and Drosophila (d) LGIC subunits included for comparison. The numbers at branch points are bootstrap values. B, lineup of ACC-1 to -4 with the serotonin-gated chloride channel MOD-1 and the rat αβ nicotinic acetylcholine receptor. The four predicted transmembrane domains (M1–M4) are underlined. The filled triangle marks the Cys loop cysteines. The two daggers mark the proline-alanine motif that determines anion selectivity. The lowercase letters (a–f) indicate the position of the six loops that form the ligand-binding site of nAChRs. The shading indicates degree of conservation.
Fig. 2. ACC-1 and ACC-2 form homomeric acetylcholine-gated channels. A and B, traces showing the response of ACC-1 and ACC-2 homomers to acetylcholine and arecoline. C and D, agonist dose-response curves to acetylcholine (A), arecoline (Δ), and atropine (○). The number of data points is indicated in parentheses. E and F, inhibitor response curves. Shown is inhibition of the response to 1 and 10 μM ACh for ACC-1 and ACC-2, respectively. ○, strychnine; △, atropine in E and dihydro-β-erythroidine in F. n = 4 for all curves in E and F. The error bars represent S.E.
not ACC-2. Cytisine, another nAChR agonist, similarly acted as a weak agonist of ACC-2 channels and as an antagonist of both ACC-1 and -2. The highest potency antagonists had the rank order: d-tubocurarine (d-TC) > strychnine > atropine > dihydro-β-erythroidine (dβe) > hexamethonium (C6) for ACC-1, and d-TC > strychnine > dβe > C6 for ACC-2 (Figs. 2 (E and F) and 4B). At 1 mM, C6 acted simultaneously as an agonist and an antagonist of ACC-1. Interestingly, 20 μM d-TC, a competitive antagonist of nAChRs and 5-hydroxytryptamine type 3 receptors, blocked ACC-1 and -2 completely. Weak activation by nicotine and cytisine and block by strychnine, d-TC, and dβe are characteristic of the vertebrate α9 nAChR channels (32, 33) and of the Aplysia ACh-gated chloride channel (3). However, unlike these channels, ACC-1 and -2 were not blocked by α-bungarotoxin (α-BT).

Although nicotinic agonists had little effect on ACC-1 and ACC-2, arecoline, an agonist of metabotropic ACh receptors, evoked a current that was 82–89% of the maximal ACh-elicited current (Fig. 2, A and C). Arecoline evoked a slowly desensitizing current in both the ACC-1- and ACC-2-expressing oocytes with an EC50 of 4.7 ± 0.11 and 754 ± 22 μM, respectively (Fig. 2, B and D). A rebound inward current was observed upon the removal of arecoline in both types of receptors, an indication of agonist-dependent open channel block (34, 35). We noted that the estimated Hill coefficients for arecoline of 2.66 ± 0.23 and 1.59 ± 0.09 for the ACC-2- and ACC-1-expressing oocytes, respectively, are not significantly different from the Hill coefficient for the response to ACh, indicating a similar degree of cooperativity of ACh and arecoline. Atropine, a nonselective antagonist of metabotropic ACh receptors and a competitive antagonist of α9 nAChRs, activated the ACC-2 channel with an EC50 of 873 ± 63 μM with an estimated Hill coefficient of 0.98 ± 0.07 (Fig. 2D) but evoked only 43% of maximal ACh-elicited current. In contrast, we detected no atropine-evoked activation (up to 1 mM) of the ACC-1-expressing oocytes. Instead, atropine blocked the response of ACC-1 channels to 1 μM ACh with an IC50 of 23 μM (Fig. 2). Thus, the pharmacological profiles of ACC-1 and -2 support a distinct ACC ligand-binding site.

**Fig. 3. ACC-1 and ACC-2 are anion channels.** A and B, I-V curves of ACC-1 and ACC-2 channels in normal (○) external solution (96 mM sodium and 104 mM chloride), low (△) sodium (0 mM), and low (▲) chloride (7.6 mM) ND96. n = 4 for all curves in A and B. C, plot of reversal potential versus external chloride concentration. Each point represents a reversal potential of an oocyte expressing ACC-1 (▲) or ACC-2 (○). The line represents the theoretical relationship predicted by the Nernst equation for a chloride-selective channel assuming 43 mM internal chloride. The error bars represent S.E.

**Fig. 4. ACC pharmacology.** A, mean activation of homomeric channels expressed in oocytes as a percentage of the response to 10 μM (ACC-2; white bars) or 1 μM (ACC-1; black bars) ACh. B, mean percentage inhibition of the response to ACh (concentrations as in A). n is indicated above each bar. The error bars represent S.E.

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**ACC-3 and ACC-4 May Form Obligate Heteromers—**Because ACC-3 and ACC-4 do not respond robustly to ACh as homomers, we considered the possibility that they form obligate heteromers with other ACh-gated chloride channel subunits. ACC-3 forms a functional heteromeric channel with ACC-1. Co-expression of ACC-1 + ACC-3 generated a channel that exhibited a pronounced desensitization compared with the ACC-1 homomer (Fig. 5A versus Fig. 2A). Moreover, the response of ACC-1 + ACC-3-expressing oocytes to ACh was more than 200-fold less potent (EC50 = 39.6 ± 1.6 μM) than that of
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homomeric ACC-1 channels. The estimated Hill coefficient of 0.78 ± 0.02 indicates that the ACC-1 + ACC-3 heteromer has fewer ACh binding sites than the ACC-1 homomer (Fig. 5B). Coexpression of ACC-1 with ACC-4 did not change significantly the maximal response, EC_{50} (0.36 ± 0.02) or Hill coefficient (1.11 ± 0.07) relative to the ACC-1 homomer (Fig. 5B). Thus, there is no indication that ACC-4 interacts with ACC-1.

Oocytes coexpressing the ACC-2 cRNA with the ACC-3 or ACC-4 cRNAs either exhibited a weak response of 50–60 nA to 1 mM ACh or did not respond, respectively (Fig. 5C); nor did ACC-2 + ACC-3; nor ACC-2 + ACC-4-expressing oocytes respond to 1 mM serotonin, GABA, glutamate, glycine, or histamine. Inhibition was specific for ACC-2, since ACC-3 and ACC-4 did not inhibit expression of a glutamate-gated chloride channel subunit, AVR-15, or expression of ACC-1 (Fig. 5C; see above). We interpreted this result as indicating that ACC-3 and -4 are able to assemble with ACC-2 in a heteromeric channel and interfere with its gating or trafficking.

**DISCUSSION**

We have identified a new family of Cys loop LGICs, the nematode ACh-gated chloride channels. We report the first molecular characterization of an anion-selective ACh receptor and show that a distinct class of Cys loop LGICs has evolved to mediate inhibitory cholinergic neurotransmission. This is also the first evidence of ACh-gated chloride channels in nematodes and suggests that fast inhibitory cholinergic neurotransmission is more widespread in the animal kingdom than previously suspected.

The ACh-gated chloride channel subunits in *C. elegans* belong to the superfamily of Cys loop ligand-gated ion channels. As such, we would predict that the ACC channels are pentameric. We showed that both ACC-1 and ACC-2 form homomeric channels when expressed in *Xenopus* oocytes but also that ACC-1 interacts with ACC-3 and ACC-2 interacts with both ACC-3 and ACC-4. The interaction of ACC-1 with ACC-3 produces a channel that could function in *vivo*, albeit one with a lower sensitivity to ACh than the ACC-1 homomer. The interaction of ACC-2 with ACC-3 and -4 is more problematic, since these subunits appear to inhibit ACC-2. We suspect that these subunits assemble into heteromers but require additional subunits to form a functional channel. However, we cannot rule out the possibility that, although capable of assembling into a heteromer, these subunits are prevented from doing so in *vivo* or that ACC-3 and -4 negatively regulate ACC-2. Determining which subunits are co-expressed in *vivo* will help resolve this issue. Finally, the ability of the ACC-3 and -4 to associate with ACC-1 and -2 is consistent with the proposition that the ACCs constitute a family of ACh-gated chloride channel subunits.

One of the most unusual features of these channels is the association of gating by ACh with anion selectivity. We can point to a clear structural motif that accounts for the anion selectivity. The M2 transmembrane domains line the pore of the channel and determine ion selectivity (30). At the cytoplasmic end of this domain is a Pro-Ala-Arg motif that is found in most anion channels in *C. elegans* (28–30).

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binding pocket are not conserved between the ACCs and the nAChRs (Fig. 1B). Most notably, the adjacent cysteines of the C loop, a hallmark of the ligand-binding nAChR α subunits, are absent from the ACCs. Thus, ACCs may have evolved to bind ACh independently of the nAChRs.

The unusual pharmacological profiles of the ACC subunits support a unique acetylcholine-binding site. That nicotine, the defining agonist of nAChRs, and cytisine, a related agonist, are weak agonists and/or antagonists of ACC-1 and -2 distinguishes the ACCs from most nicotinic receptors. The weak nicotine response is not, however, unique. The *C. elegans* levamisole receptors (nAChRs) are insensitive to nicotine, and it has been shown that *C. elegans* has both nicotinic-sensitive and -insensitive cation-selective ACh receptors (38). Moreover, nicotine and cytisine are antagonists of the vertebrate α9 nAChRs (33). More surprising is that arecoline is an efficient agonist of both ACC-1 and -2, although with substantially lower affinity than ACh. Arecoline has been postulated to act on cation-selective nematode and insect LGICs, so this sensitivity to arecoline may be a common feature of invertebrate ACh receptors (39, 40). Finally, α-BT, which is selective for vertebrate α7 and α9 nAChRs in the central nervous system and the nAChRs of the neuromuscular junction, does not block the ACC channels. α-BT binds primarily to the C loop in nAChRs (41). Therefore, the lack of effect on ACC channels presumably reflects the inability of α-BT to bind the ACC subunit’s divergent C loop sequences.

Are the *Aplysia* ACh-gated ion channels orthologs of the ACCs? There are pharmacological similarities that suggest they might be (3). One of the two *Aplysia* channels identified desensitized slowly, a characteristic of ACC-1 homomers and ACC-1/ACC-3 heteromers. Nicotine and cytisine were also poor agonists of the slowly desensitizing *Aplysia* channel. Potencies of the antagonists for nematode and mollusk receptors were in the same range. However, *Aplysia* receptors appear to have an EC50 for ACh of >100 μM. Perhaps most importantly, the *Aplysia* channels were blocked by α-BT, indicating that they share a much greater similarity to the nicotinic receptors, at least in the C loop, than do the ACC subunits. Ultimately, whether the *Aplysia* channels evolved independently will have to be determined by sequence analysis.

Economically important antiparasitic nematocides target Cys loop LGICs. Levamisole is an agonist of a subset of nicotinic-type receptors (42), and ivermectin activates glutamate-gated ion channels (43). Levamisole is an agonist of a subset of nicotinic-type receptors (42), and ivermectin activates glutamate-gated ion channels (43). Therefore, the lack of effect on ACC channels presumably reflects the inability of α-BT to bind the ACC subunit’s divergent C loop sequences.

**Acknowledgment**—We thank E. Cooper for critical reading of the manuscript.

**REFERENCES**

1. Kehoe, J. (1972) *J. Physiol.* 235, 85–114
2. Kehoe, J. (1972) *J. Physiol.* 235, 115–146
3. Kehoe, J., and Mcintosh, J. M. (1998) *Neurosci. 18*, 8189–8213
4. Unwin, N. (1990) *Nature 343*, 37–43
5. Hille, B. (1992) *Ionic Channels of Excitable Membranes*, 3rd Ed., pp. 405–422.