Introduction to Thematic Minireview Series on Celebrating the Discovery of the Cysteine Loop Ligand-gated Ion Channel Superfamily

F. Anne Stephenson
From the University College London School of Pharmacy, London WC1N 1AX, United Kingdom

The year 2012 marks the 25th anniversary of the discovery of the Cys loop ligand-gated ion channel superfamily of neurotransmitter receptors. This minireview series celebrates this with a series of articles reviewing current information for each of the family members, nicotinic acetylcholine receptors, glycine receptors, GABAa receptors, serotonin-3 (5-HT3) receptors, and glutamate-gated chloride ion channels of proteasome invertebrate phyla.

Communication and subsequent information processing between excitable cells are a result of neurotransmission. By the late 1960s, the molecular components of fast neurotransmission were beginning to be identified, at least for the neurotransmitter acetylcholine, at the neuromuscular junction. Thus, it was established that acetylcholine was released from synaptic vesicles and diffused across the synaptic cleft, where it bound to a specific receptor site on the membrane. The outcome of this binding was a subsequent change in the permeability properties of the membrane for Na+ and K+ ions, resulting in membrane depolarization and propagation of the action potential. The molecular nature of this “receptor” was unclear, but fortuitous natural phenomena contributed to the identification of this receptor as an integral membrane protein, the nicotinic acetylcholine receptor. First, there was the realization that the electric organs of the electric ray and the electric eel communicated via nicotinic cholinergic synaptic transmission, yielding a rich source of the receptor for purification. Second was the discovery of the mode of action of α-neurotoxins isolated from the venom of certain snakes. These were shown to bind with high affinity to nicotinic acetylcholine receptors, and importantly, they were used for receptor isolation via affinity chromatography. Reconstitution of the purified receptor into liposomes revealed that the receptor and the ion channel that opened in response to nicotinic acetylcholine receptor activation were one and the same, the nicotinic acetylcholine receptor ligand-gated ion channel (1).

As progress in the identification of the nicotinic acetylcholine receptor was being made, in parallel studies, evidence was accumulating to establish the role of other molecules as fast-acting neurotransmitters, which, by analogy with acetylcholine, were proposed to mediate their effects via binding to their cognate receptors, two examples being the inhibitory neurotransmitters glycine and γ-aminobutyric acid (GABA). These neurotransmitter receptors were hard to study because they did not have the advantages that aided the purification of nicotinic receptors. There were no high affinity neurotoxins and no abundant source to facilitate isolation. However, in the 1970s, radioisotopes became available that enabled the synthesis of small molecules with high specific radioactivity. This, coupled with the development of radioligand binding assays utilizing these radioactive tracers and based on methods forged in the cholinergic (1) and also, by Lefkowitz et al., the β-adrenergic (2) receptor fields, gave a means for biochemists to be able to detect, to quantify, to detergent-solubilize with the retention of activity, and ultimately, to purify neurotransmitter receptors. The isolation to apparent homogeneity of both glycine (3) and GABAa (4) neurotransmitter receptors was reported in the Journal of Biological Chemistry.

Concurrently, a major development that transformed not only the ligand-gated ion channel receptor field but the study of channels per se was the work of Eric Barnard and Ricardo Miledi in London, United Kingdom. Following the pioneering studies of John Gurdon in Cambridge, United Kingdom, utilizing the Xenopus oocyte to translate microinjected messenger RNA molecules (5), they exploited this system such that mRNA isolated from an appropriate source, i.e. brain, was translated in the oocyte to form functional ion channel receptors (6). These expressed receptors were readily analyzed by electrophysiological techniques. (Note that oocyte expression was pivotal for the cloning of some neurotransmitter receptor genes for which protein information was intractable.)

It was the culmination of all of this progress together with advances in molecular cloning technology that resulted in 1987 in the publication of two landmark Nature articles that described the identification of genes encoding glycine (7) and GABAa (8) receptor subunits. The deduced primary structures of these key proteins of the central nervous system revealed, perhaps not surprisingly because both were known to be ligand-gated chloride ion channels, that they were homologous. What was unexpected, however, was that both proteins shared amino acid sequence similarity, notably a conserved Cys-Cys loop in their respective N-terminal domains, with the well-characterized, excitatory nicotinic acetylcholine receptor, a cation channel. Thus, the superfamily of Cys loop ligand-gated ion channels was uncovered.

The year 2012 marks the 25th anniversary of these groundbreaking papers. This minireview series celebrates this with a series of articles reviewing current information for each of the family members, nicotinic acetylcholine receptors, glycine...
receptors, GABA_\textsubscript{A} receptors, serotonin-3 (5-HT\textsubscript{3}) receptors (initially cloned by David Julius’ group in San Francisco by screening a neuroblastoma expression library for functional expression of serotonin-gated currents in \textit{Xenopus} oocytes (9)), and glutamate-gated chloride ion channels that are found in proteasome invertebrate phyla and that, similar to 5-HT\textsubscript{3} receptors, were also cloned via functional expression (10).

A direct result of the cloning studies was to reveal an unexpected heterogeneity of neurotransmitter receptors. While a formidable challenge to biochemists to determine the actual subunit composition and function of native \textit{in vivo} receptors, this was a boost for many drug discovery programs. Indeed, neurotransmitter receptor heterogeneity is currently being exploited by industry to identify novel subtype-selective agonists, antagonists, and allosteric effectors for the treatment of many central nervous system disorders, including the dementias, in which these important brain proteins are implicated. Furthermore, the structures of the full-length glutamate-gated chloride ion channel (11) and the full-length prokaryotic \textit{Erwinia chrysanthemi} receptor, a cation channel gated by GABA (12), recently solved at the atomic level will serve as templates for the other members of the family in their native, agonist-, antagonist-, or allosteric effector-bound states such that the molecular mechanisms of channel opening can be elucidated at the atomic level and ultimately permit rational drug design.

Acknowledgments—I thank most sincerely all of the authors for their contributions: Jean-Pierre Changeux, a founding father of receptor biochemistry and allosteric mechanisms; Heinrich Betz and Erwin Sigel, whose early work on glycine and GABA_\textsubscript{A} receptor structures, respectively, is the subject of the accompanying Classics; and Adrian Wolstenholme and Sarah Lummis, glutamate-gated chloride and 5-HT\textsubscript{3} channel aficionados, respectively.

REFERENCES

2. Lefkowitz, R. J., Sharp, G. W., and Haber, E. (1973) Specific binding of \beta\textsubscript{-}adrenergic catecholamines to a subcellular fraction from cardiac muscle. \textit{J. Biol. Chem.} 248, 342–349