Acetylcholine Receptors: Too Many Channels, Too Few Functions

Lucia Sivilotti and David Colquhoun

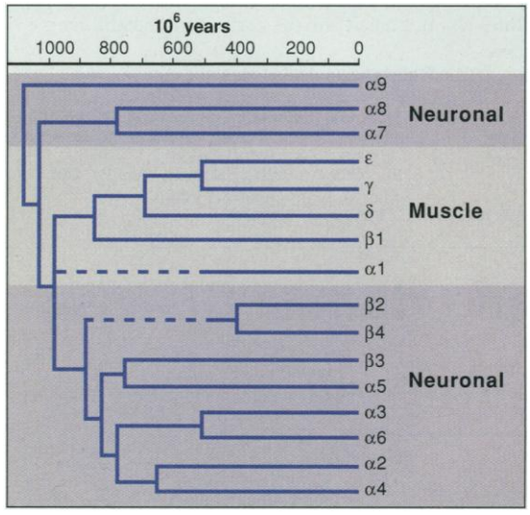
Paradoxically, the combined power of patch clamping and of molecular genetic methods has caused a return to an age of stamp collecting. At almost daily intervals new ion channels are cloned and characterized, but in many cases evidence for their physiological importance is flimsy or nonexistent. The best known of all receptors—the nicotinic acetylcholine receptor—provides a good example.

The nicotinic receptor in muscle and ganglia was the first ligand-gated receptor to be identified (by Langley, who 90 years ago coined the term "receptor" in reference to it); it was the receptor that inspired the first derivation of the Langmuir equation by A. V. Hill (*1*); it was the first receptor shown to mediate transmission at chemical synapses (by Dale and his colleagues in the 1930s); and it was the first to be purified and cloned (in the 1980s) (*2*). The nicotinic acetylcholine receptor provided the first detailed description of fast synaptic transmission (largely by Katz and his colleagues) (*2*), the first recordings of single-ion channels, and the first detailed kinetic analysis (*3*). At the molecular level, this receptor has the best-described three-dimensional structure (*4*).

From 1951 (*5*) onward, it became clear that the nicotinic receptor in autonomic ganglia was not quite the same as that on skeletal muscle fibers, but apart from that, all was simple. Fast nicotinic synapses were supposed to be absent from the brain (except at Renshaw cell synapses, which are formed in the spinal cord by collaterals of motor axons), the main fast transmitter in the central nervous system (CNS) being glutamate. The brain does have neurons that contain and release acetylcholine, but this acetylcholine was thought to act on the relatively slow, GTP-binding protein (G protein)–coupled muscarinic receptors. Then, in 1986, results obtained with in situ hybridization suggested that, quite unexpectedly, nicotinic receptors were abundant in many parts of the brain (*6*). What are they doing there? As yet, nobody really knows, because fast nicotinic synapses still cannot be found in the brain. But a new report by McGehee and co-workers in this issue of *Science* (*7*) sheds new light on the question by showing that nicotine enhances excitatory transmission in the CNS by acting on presynaptic nerve endings to increase transmitter release.

The authors are in the Department of Pharmacology and at Wellcome Laboratory for Molecular Pharmacology, University College London, London WC1E 6BT, UK.

Nicotinic receptors from muscle are oligomers of five subunits, which surround a central pore. Binding of acetylcholine causes the pore (ion channel) to open within microseconds—and this speed is, inter alia, what makes it possible to play the Waldstein sonata. The muscle receptors consist of α1, β1, γ, ε, and δ subunits (*6*), and the neuronal receptors are made up of some combination (still largely unknown) of α2 to α9 and β2 to β4 subunits, although there may be others yet undiscovered (see figure). The confusion in the field is exacerbated by the fact that the chick and rat receptors are not identical.



Evolutionary tree of nicotinic acetylcholine receptor subunits. [Reproduced with permission (*15*)] A somewhat different version is given by Ortells and Lunt (*16*).

A few facts are clear. Most neuronal nicotinic receptors in the brain are made up of two or more different sorts of subunits; only α7, α8, and α9 can form efficient channels homomerically (although the native α7-containing channels may not be homomeric). In addition, the α7 to α9 channels are the only neuronal nicotinic receptors that are sensitive to the snake toxin α-bungarotoxin, which has the ability to block irreversibly the muscle-type receptor (*6*). Finally, the brain contains many "high-affinity nicotine-binding" sites and many α-bungarotoxin–binding sites, but these sites are clearly not the same (*8*). The nicotine-binding sites probably represent channels formed predominantly from α4 and β2 subunits, whereas the α-bungarotoxin sites are likely to be the α7-type channels, which are not involved in fast synaptic transmission (*8*).

Why should we be interested in nicotinic receptors in the brain, apart from the fact that they are there? There are three obvious reasons, all in areas where it is not easy to separate the hard facts from the hype and the grantsmanship. First, it is generally supposed that these receptors must have something to do with tobacco addiction. Second, nicotine seems to improve performance in some learning tasks. And third, there is a loss of "high-affinity nicotine-binding sites" in the brains of patients suffering from Alzheimer's disease (*9*). This last finding does not, of course, mean that the cause of the disease has anything to do with nicotinic receptors, but it has led to the hope (for which the evidence so far is dubious) that nicotinic agonists might provide a symptomatic treatment. How might these effects be related to nicotinic receptors?

There are two ways in which chemicals may influence the brain. The fast synaptic transmitters (mainly glutamate) mediate propagation of impulses from one cell to another like that at peripheral synapses. At such synapses, the increase in transmitter concentration is very brief and very localized. Second, there is what might be termed the "soup" theory of the brain: The brain functions in the presence of an ambient mixture of regulatory molecules (such as dopamine, acetylcholine, serotonin, glycine, inorganic ions, and peptides), which are not fast transmitters, but which regulate the excitability of postsynaptic cells and, by presynaptic actions, the amount of the primary transmitter released at synapses. The exact composition of the soup is likely to vary with time, position, and local neuronal activity, providing a form of local regulation. If synapses are missing or damaged, as occurs in Alzheimer's disease, it is unlikely that flooding the system with an analog (like nicotine) of a fast transmitter could do much to help damaged primary transmission, but it is not unreasonable to think that adjustment of the composition of the "soup" might help.

The report by McGehee *et al.* goes a long way to justify this view. They show convincingly that low concentrations of nicotine, comparable with those in the blood of smokers, can increase release of transmitters, both glutamate in the brain and acetylcholine in autonomic ganglia (most

other studies have used higher nicotine concentrations). They show, with the help of careful controls, that this is a direct presynaptic action. Another contribution of this work is the demonstration that the presynaptic receptor is likely to contain the α7 subunit, because the nicotine effect can be blocked by α-bungarotoxin in control conditions, but not after the α7 subunit is eliminated with antisense treatment. In addition, McGehee *et al.* find that nicotine causes an increase in intracellular calcium concentration in presynaptic endings. This observation may explain the effect of nicotine on fast transmission, because homomeric α7 channels have a rather high calcium permeability (*10*). The authors suggest that such presynaptic actions may underlie the behavioral and cognitive effects of nicotine.

Nicotinic enhancement of the spontaneous release of the neurotransmitters γ-amino butyric acid (GABA) and dopamine and of evoked excitatory synaptic transmission in the brain has been reported previously (*11*). But the work of McGehee *et al.* differs in that it provides the first strong evidence for the involvement of α7-containing channels. Other reported cases of presynaptic nicotine effects in the CNS are not sensitive to α-bungarotoxin (*11*, *12*). Most other pre- or postsynaptic nicotine actions also seem to require higher nicotine concentrations (*11*, *13*), although it is puzzling that the potency of nicotine on recombinant α7 receptors is reported not to be very high in absolute terms (*14*). It is also interesting that the "high-affinity nicotine-binding" areas, rather than the α-bungarotoxin–binding areas, are primarily affected in Alzheimer's patients (*9*). One remaining question for all studies is whether the nicotine receptors are ever actually exposed to acetylcholine in real life. This has never been demonstrated, although the presence of neurons containing cholineacetyltransferase makes the possibility plausible.

If most of the important actions of nicotine in the brain are presynaptic effects through α7-containing receptors, what are all the other subunits there for? Nobody knows at present. It is not considered respectable in polite company to suggest that these other subunits might constitute a redundant evolutionary hangover, although one cannot help thinking of the decades that were spent looking for the physiological function of the vast number of histamine receptors in the body. That problem was never really solved—people just got bored with it. We can only hope that the same fate does not await the α4-containing receptors.

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Minisatellites and Human Disease

Theodore G. Krontiris

The appearance of unstable DNA sequences in key regions of the human genome evokes the image of a mischievous Nature casually dropping a box of matches within reach of an adventuresome and unattended child. The predictable consequences emerge in the dramatic example of the unstable trinucleotide repeats: a brushfire of disease-producing mutations in fragile X syndrome, myotonic dystrophy, Huntington's disease, and a growing host of other genetic disease syndromes (*1*). Now evidence is accumulating from studies of the insulin (*INS*) and Ha-ras (*HRAS1*) loci that another class of repetitive sequences, hypervariable minisatellites, contributes a subtler, but potentially more widespread, influence on the heritable risk of disease.

Minisatellites are tandem arrays of a locus-specific consensus sequence that varies between 14 and 100 base pairs (bp) in length (*2*). Such structures are often polymorphic in the number of tandem repeats of the consensus [hence, the alternative designations, variable number of tandem repeats (VNTRs) or variable tandem repetitions (VTRs)]. Dispersed throughout the human genome (and likely those of all vertebrates), minisatellites are often situated just upstream or downstream of genes; many occur within introns. The *INS* VNTR is 600 bp upstream of the transcriptional start site (*3*), and the *HRAS1* minisatellite is 1000 bp downstream of the polyadenylation signal (*4*).

VNTRs are extraordinarily hyperallelic; many loci display dozens of alleles. As a consequence, the heterozygosity rate (het rate), or fraction of individuals in the population with two different alleles, can approach 100%. This means a geneticist may screen an auditorium full of compliant colleagues and never find a homozygote at many VNTR loci. The *INS* minisatellite has a het rate in excess of 90%. Curiously, the *HRAS1* minisatellite displays a het rate of only 65%.

The driving force underlying this genetic plasticity is, of course, a mutation rate that can exceed 10% per gamete (*5*). We are only beginning to understand the mutational processes giving rise to such instability. The intuitively obvious mechanism, single crossovers at the site of slippage and mispairing of tandem repeats, probably occurs infrequently, if at all (*6*). Instead, complex internal rearrangements of the minisatellite appear in new mutations, almost exclusively at one end of the tandem array (*7*). Analysis of the DNA sequence indicates that this process involves interallelic exchange, implicating gene conversion (*7*). The rate of mutation varies from locus to locus and from individual to individual at a given locus (*5*, *8*). The human minisatellite MS32 possesses a cis-acting promoter of mutation on one flank, an observation, if reproduced at other VNTRs, that could explain both the varying rate and the polarity of the mutational mechanism. In addition, some of the

The author is in the Department of Medicine and Program in Genetics, Tufts University School of Medicine and New England Medical Center Hospitals, Boston, MA 02111, USA.