receptor conformation which is characterized by inward movements of three of the four helixes forming the site compared to the inactive conformation. This structural difference explains why competitive antagonists of the mAChRs generally are larger molecules than orthosteric agonists (Figures 16.7 and 16.9b). The aforementioned difficulties obtaining subtype-selective orthosteric ligand are rooted in the complete conservation of the residues forming the orthosteric sites in the five subtypes. Conversely, the pronounced subtype-selectivity displayed by allosteric and bitopic ligands comes from these ligands targeting less conserved receptor regions.

The structural diversity of allosteric mAChR ligands (Figure 16.8) suggests that these ligands potentially could target several different sites in the receptor. However, extensive research into the modes of action of the first generation of allosteric modulators as well as AC-42 (**16.36**) and BQCA (**16.39**) indicates that most of these modulators target a common allosteric site located superficially to the orthosteric site in the mAChR. The existence of such a common allosteric site has been further supported by a recent crystal structure of M_2 in complex with iperoxo (**16.23**) and LY2119620 (**16.41**), in which the PAM is observed to bind to a site just above the orthosteric site and in this way facilitate the stabilization of the active mAChR conformation mediated by the orthosteric agonist (Figure 16.9a and d). Other PAMs acting through this common allosteric site most likely potentiate mAChR signaling through similar mechanisms, and conversely binding of a ligand to this site could be envisioned to favor the inactive rather than the active receptor conformation and in this way act as a NAM.

The mAChR PAMs (including **16.39**–**16.43**) obviously target site(s) in the receptors topographically distinct from the orthosteric site since they require concomitant binding of agonist to the receptor in order to mediate their effects. In contrast, several of the identified M_1-selective agonists (including **16.36**–**16.38**) have been proposed to be bitopic ligands, meaning that they target the orthosteric site as well as an allosteric site in close proximity to it. This hypothesis is supported by the structural resemblance that fragments of these agonists bear to orthosteric mAChR ligands, whereas other parts of the agonists are expected to protrude from the orthosteric site into neighboring receptor regions. In this scenario, these agonists obtain their pronounced subtype-selectivity from less conserved allosteric site while their interactions with the orthosteric site contribute to their binding affinity. The binding of some of the bitopic mAChR agonists have been speculated to bridge between the orthosteric site and the common allosteric site located just above it in the mAChR (Figure 16.9a).

16.5 NICOTINIC ACh RECEPTORS

The nAChRs are members of the Cys-loop receptor superfamily that also comprises ligand-gated ion channels for GABA, glycine, and serotonin (Chapter 12). The nAChR is a membrane-bound protein complex composed of five subunits forming an ion pore through which cations (Na^+, K^+, and Ca^{2+}) can enter the cell when the receptor is activated, resulting in depolarization of the neuron. The 16 human nAChR subunits assemble into "muscle-type" and "neuronal" receptor subtypes (Figure 16.10a and b). The muscle-type nAChR composed of α_1, β_1, δ, and γ/ε subunits is localized postsynaptically at the neuromuscular junction, where it mediates the electrical transmission across the anatomical gap between the motor nerve and the skeletal muscle, thus creating the skeletal muscle tone. The neuronal nAChRs are heteromeric or homomeric complexes assembled from α_2–α_7, α_9, α_{10}, and β_2–β_4 subunits (Figure 16.10b). The high heterogeneity of native neuronal nAChR populations thus arises from the differential expression of these subunits in the CNS combined with their ability to be incorporated into a substantial number of receptor combinations, each characterized by distinct functional and biophysical properties (Figure 16.10c). This molecular diversity extends down to the specific α/β combination, as exemplified by the two $\alpha_4\beta_2$ receptors stoichiometries $(\alpha_4)_2(\beta_2)_3$ and $(\alpha_4)_3(\beta_2)_2$, that exhibit very different agonist sensitivities and desensitization properties (Figure 16.10c). The neuronal nAChRs are located at presynaptic and postsynaptic densities in autonomic ganglia and in cholinergic neurons throughout the CNS, and equally important to