The complexity of AChE function is reflected in the diverse mechanisms by which different AChEIs exert their inhibition of the enzyme. The "carbamoylating" inhibitors (**16.5**–**16.8**) are actually substrates for AChE as they all contain carbamate groups that, analogously to the ester group in ACh, can be hydrolyzed by the enzyme, thus yielding the respective carbamate and stigmine (**16.5**–**16.7**) or dimethylamino-α-methylbenzyl (**16.8**) moieties of the compounds (Figure 16.6b). However, in contrast to the very fast (microseconds) cleavage of acetate from the Ser^{200} residue following the hydrolysis of ACh, the dissociation of the carbamate group from Ser^{200} is very slow (seconds–minutes). Thus, these AChE substrates exert *de facto* inhibition since the catalytic site is unable to bind and hydrolyze ACh as long as the carbamate group is covalently bound to Ser^{200} (Figure 16.6b). Thus, the kinetics of the inhibition mediated by these inhibitors is mainly determined by the stability of the formed Ser^{200}-carbamate conjugate which varies somewhat for the different carbamate groups.

In contrast to the carbamoylating inhibitors, the other reversible AChEIs (**16.3**–**16.4**, **16.9**–**16.15**) are "true" inhibitors in the sense that they are not hydrolyzed by AChE but inhibit its function through binding to various sites. Inhibitors like tacrine (**16.3**) and galanthamine (**16.9**) interact with residues both in the catalytic anionic site and the esteratic subsite and thus compete directly with ACh for binding to the catalytic site (Figure 16.5b). On the other hand, donepezil (**16.4**) targets the gorge connecting the catalytic site with surface of the enzyme, and the dimethoxy**–**indanone and benzyl piperidine moieties of the inhibitor interact with residues in peripheral anionic site and in the catalytic anionic site, respectively (Figure 16.5b). Propidium (**16.15**) and fasciculin**-**2, a peptide toxin found in the venoms of mamba species, inhibit AChE function noncompetitively by binding exclusively to the peripheral anionic site, thus prohibiting the substrate from gaining access to the catalytic site. Finally, the remarkably high inhibitory potencies displayed by bivalent ligands such as **16.12** have been attributed to their ability to form interactions with both the peripheral anionic site and the catalytic site in the AChE.

Irreversible organophosphorus AChEIs are esters or thiols derived from phosphoric, phosphonic, phosphinic, or phosphoramidic acids (e.g., **16.16** and **16.17**). Just as the carbamoylating inhibitors, these compounds are structural analogs to ACh and thus undergo similar initial interactions with the Ser^{200} residue in the esteratic subsite. This gives rise to the formation of an extremely stable covalent phosphorus-conjugate complex and a completely irreversible inhibition of enzyme function (Figures 16.5b and 16.6c). Thus, the toxicity of these inhibitors is rooted in the fact that normal AChE function in the body only can be restored by resynthesis of the enzyme. The formation of the phosphorus AChE conjugate can be reversed with oxime-based "reactivators," such as **16.18** (Figure 16.6c), and mAChR antagonists such as atropine have also been used to treat organophosphorate poisoning.

16.4 MUSCARINIC ACh RECEPTORS

The mAChRs belong to class A of the G-protein coupled receptor (GPCR) superfamily (Chapter 12). Thus, the mAChR component of cholinergic signaling is mediated by their coupling to G-proteins and other intracellular proteins (such as β-arrestins) and the resulting downstream effects arising from second messenger cascades, modulation of ion channels, and activation of various kinases. The five mAChR subtypes, termed M_1–M_5, are ubiquitously expressed throughout the CNS and in most peripheral tissues. The physiological functions mediated by the different subtypes have for decades been studied using knockout mice strains (where the expression of one or several mAChR subtypes has been eliminated), and in recent years these explorations have been further aided by the availability of truly subtype-selective pharmacological tools for the receptors (see later text). Whereas the M_1, M_4, and M_5 receptors predominantly are expressed in the CNS, M_2 and M_3 are found both centrally and in the periphery, including cardiac and smooth muscle tissues. An important realization to come from these studies is that many of the adverse effects produced by nonselective mAChR ligands or by AChEIs are attributable to the peripheral M_2 and M_3 receptors. These findings