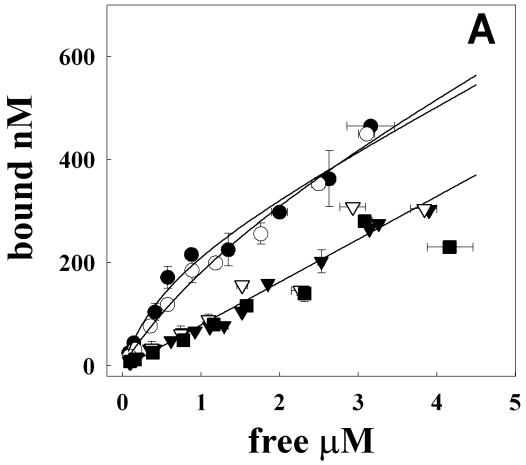
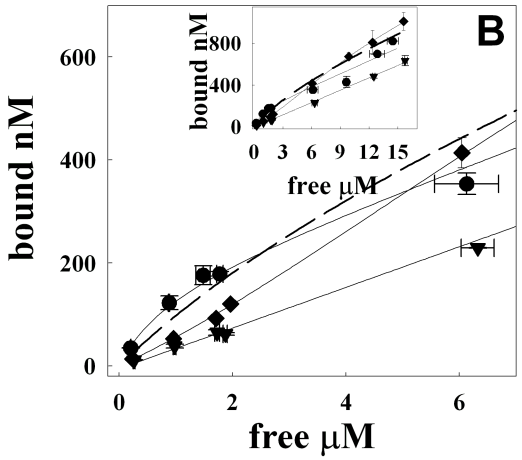

A
bound nM
600
400
200
0
0
1
2
3
4
5
free μM


B
bound nM
600
400
200
0
0
2
4
6
free μM
bound nM
800
400
0
0
3
6
9
12
15
free μM

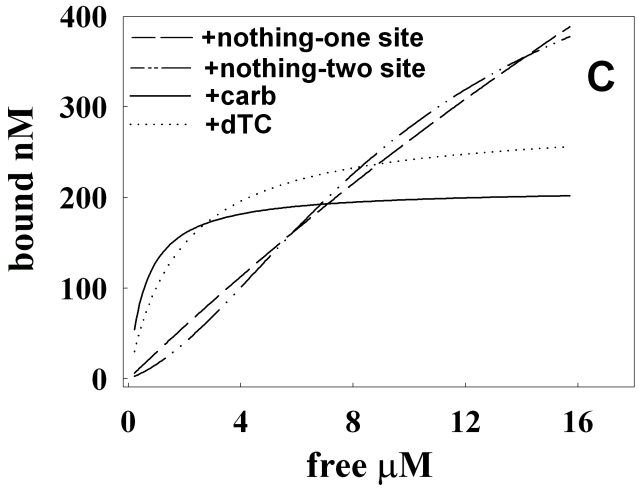

C
+nothing-one site
+nothing-two site
+carb
+dTC
bound nM
400
300
200
100
0
0
4
8
12
16
free μM

Figure 2-1. Binding of [^{3}H]ethidium in the presence and absence of various cholinergic drugs.

A, B, nAChR-rich membranes (2 mg/ml with ~900 nM ACh binding sites) were equilibrated with various cholinergic drugs (2 mM carbamylcholine (●), 100 μM dTC (○), 2 mM carbamylcholine with 100 μM PCP (▼), 10 μM αBgTx (▽), 125 μM ethidium (■), and no other drug (◆)) with increasing concentrations of [^{3}H]ethidium. After a two hour incubation, the concentration of bound and free [^{3}H]ethidium was determined by filtration. The points plotted are the averages of duplicate samples. The observed binding in the presence of αBgTx, ethidium, or carbamylcholine with PCP was fit to a line to determine the nonspecific binding (B_{ns}). The specific binding in the presence of carbamylcholine or dTC was determined according to Equation 1 in Methods. Both the B_{max} and the K_{eq} were variables, and B_{ns} was determined from the slope of the binding in the presence of carbamylcholine and PCP. The specific binding in the absence of other drugs was determined either as described for the binding in the presence of carbamylcholine or dTC or by a fit to Equation 2 in Methods. For Equation 2, both K_{eq}s were variables, as was B_{max}. However, in this model the B_{max} was assumed to be equal for the two binding sites. A. +carb: K_{eq}=0.6 ± 0.3 μM, B_{max}=200±30 nM; +dTC: K_{eq}=1.7±0.3 μM, B_{max}=270±50 nM. If [^{3}H]ethidium is constrained to a common value of B_{max}, 220 nM, then K_{eq} (+carb)=0.7 ± 0.1; K_{eq} (+dTC)=1.1 ± 0.1. +carb/+PCP: slope=0.083±0.004; +ethidium: slope=0.065±0.008; +αBgTx: 0.085±0.004 B. +carb: K_{eq}= 1.6±0.9 μM; B_{max}=210±30 nM; +nothing: Equation 1 with B_{max}=200 μM: K_{eq}=1.7±2.0 μM (dashed line); Equation 2: K_{eq1}=25±8 μM; K_{eq2}=4.2±2.3 μM; B_{max}=280±30 nM C, The specific binding in each condition was calculated from Equation 1 or Equation 2 (for the binding in the presence of no other drug). When the binding data in the absence of other drugs was determined by a fit to Equation 1, K_{eq}=100±90 μM, B_{max}=2800±2300 nM.