



Figure 3-3. Dependence of [³H]3-azidoctanol incorporation into α -subunit on the concentration of carbamylcholine.

nAChR-rich membranes (100 μ g at 2 mg/ml) were equilibrated with 1 μ M [³H]3-azidoctanol in TPS in the presence of varying concentrations of carbamylcholine and irradiated at 365 nm for 10 minutes. Samples were subjected to SDS-PAGE and visualized by Coomassie Blue. Bands corresponding to nAChR α -subunit were excised and ³H incorporation was quantified by scintillation counting. The data were fit to a

single site model $B = \frac{B_A}{1 + \frac{K}{L}} + B_0$ where B was the observed ³H incorporation at each

concentration, L was the concentration of carbamylcholine, B_A and B₀ were the agonist dependent and independent incorporation, respectively, and K was the apparent dissociation constant for carbamylcholine. K, B_A, and B₀ were varied. Based on the data, B_A=23100 \pm 1400 cpm; B₀=6900 \pm 1300 cpm; K=4.2 \pm 1.0 μ M