



Figure 2-4. Photoincorporation of [³H]ethidium diazide into integral and peripheral membrane proteins of nAChR-rich membranes in the presence of various cholinergic drugs.

nAChR-rich membranes (100 µg) were equilibrated with [³H]ethidium diazide in TPS (2 mg/ml) in the presence of 10 mM oxidized glutathione in the presence of 2 mM carbamylcholine, 2 mM carbamylcholine and 100 µM PCP, 10 µM αBgTx, 100 µM d-tubocurarine, or no other drug. After photolysis at 265 nm for 30 seconds, samples were subjected to SDS-PAGE and visualized by Coomassie Blue. Bands corresponding to indicated polypeptides, as well as a 90 kD band, containing the α-subunit of Na⁺/K⁺ ATPase, were excised. ³H was quantified by scintillation counting. Values are the average from duplicate lanes for each condition, with the ³H normalized with respect to that in the 90 kD band, to reduce variability between lanes. Average incorporation in polypeptides labeled in the presence of carbamylcholine (+/-) or the presence of carbamylcholine and PCP (+/+): α-subunit: +/-: 2098 ± 27 cpm; +/+ : 1278 ± 310 cpm. δ-subunit: +/-: 1186 ± 15 cpm; +/+ : 740 ± 159 cpm. β-subunit: +/-: 952 ± 44; +/+ : 775 ± 161. For αNK: +/-: 942 ± 84; +/+ : 1154 ± 256.