



Figure 4-3. Proteolytic mapping of sites of [³H]progesterin aryl azide incorporation into the nAChR α -subunit using *S. aureus* V8 protease.

nAChR-rich membranes (400 μ g) were labeled with [³H]progesterin aryl azide in the presence of 1 mM oxidized glutathione and in the absence of other cholinergic drugs (lanes 1 and 2) or in the presence of 100 μ M tetracaine (lane 3) or 2 mM carbamylcholine (lane 4). One sample labeled in the absence of other drugs was treated with EndoglycosidaseH (lane 2) as described in methods, while the other samples incubated in buffer without EndoglycosidaseH overnight. After incubation, membranes were submitted to SDS-PAGE, and the α -subunit was excised. The excised bands were transferred to the well of a 15% mapping gel and digested with V8 protease as described in methods. A, mapping gel stained with Coomassie Blue. B, fluorogram of mapping gel, exposed for 4 weeks. The mobility of the proteolytic fragments is indicated on the left. Shown above is a schematic indicating the positions of the four fragments within the primary structure of the nAChR α subunit. Based on scintillation counting of parallel lanes, the cpm incorporated in the absence of carbamylcholine was: α V8-20: 911; α V8-18: 498; α V8-10: 6749.