SYNAPTIC JUNCTIONAL GLYCOCONJUGATES FROM CHICK BRAIN
Glycoprotein Identification and Carbohydrate Composition

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Forebrains from day-old chicks were homogenized and fractionated by differential sedimentation and density gradient centrifugation to yield subcellular fractions. The synaptosomal plasma membrane fraction was further treated with Triton X-100 to yield subsynaptic membrane fractions including synaptic junctions. Glycoproteins from these subsynaptic membrane fractions were identified after separation by SDS-polyacrylamide gel electrophoresis by incubating the gel slabs with radioiodinated concanavalin A. Two lectin-binding proteins were discerned in the synaptic junction fraction while none were observed in the Triton-soluble portion of the synaptic plasma membrane. The carbohydrate content of the glycoproteins from each subcellular fraction was quantitated after methanolysis and derivatization as o-methyl-trifluoroacetyl analogs by gas-liquid chromatography. The lowest concentration of glycoprotein sugars was found in the synaptic junction, mitochondrial, and soluble fractions while the greatest concentration was found in the myelin, light-synaptic plasma membrane, and the Triton-soluble portion of the synaptic plasma membrane. Of the subcellular fractions, the synaptic junction contained the highest proportion of mannose and lowest proportion of sialic acid. Moreover, this fraction's content of galactose and N-acetylglucosamine, relative to mannose was the lowest while its content of fucose was low. The oligosaccharide chains extending into the synaptic cleft therefore are predominantly of the “neutral, mannose-rich” type and are attached to a limited number of high-molecular-weight glycoproteins.

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INTRODUCTION

Several roles have been suggested for cell surface glycoproteins and glycolipids. These include cell–cell recognition (47), contact inhibition (1), cell growth, and substrate adhesion (56). In the nervous system, glycoconjugates in the synaptic membranes have been implicated in the formation and maintenance of appropriate synaptic contacts (2, 4, 5, 12). For a review of the possible functions of synaptic glycoconjugates, see reference 30. In support of these arguments, the synaptic membrane has been reported to be relatively enriched in glycoconjugate carbohydrate (12) with a marked increase occurring at the time of synaptogenesis (3, 21, 35). Several hypotheses concerning how synaptic activity and the structure of the synaptic junctional oligosaccharide chains might covary have been advanced (4, 7, 8, 12, 44). It is important to know then, the sugar composition of these synaptic junctional glycoproteins, the types of oligosaccharide chains which they contain, and the number and size of peptide cores to which these chains are attached. A comprehensive review of glycoconjugates of the nervous system (11) has recently been published.

We have undertaken a compositional analysis of the glycoprotein sugars using gas–liquid chromatography and have separated the glycoproteins into molecular-weight classes by polyacrylamide gel electrophoresis. Contrary to expectations, the forebrain synaptic junctions were found to contain the least amount of protein-bound sugar relative to other subcellular fractions. Furthermore, the ratio of mannoglycopeptides (which contain N-acetylglucosamine and mannose) to sialoglycopeptides (which contain, in addition, fucose, galactose and N-acetylneuraminic acid) is highest in the synaptic junction. Nevertheless, the concentration of glycoproteins with receptors for the lectin concanavalin A (oligosaccharides derived from mannoglycopeptides) is reduced in this region. Our experimental animal is the neonatal chicken which at one day old is neurologically mature.

EXPERIMENTAL PROCEDURE

**Materials.** Concanavalin A (Con A), Sigma grade IV, was iodinated with $[^{131}]$I by the method of Greenwood and Hunter (24). Free $^{131}$I and inactive Con A were separated from active $[^{131}]$ICon A on a column of Sephadex G-50 followed by dialysis. The viability of the $[^{131}]$ICon A was ascertained by agglutination of mouse erythrocytes. Materials for acrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) were the same as used previously (54). Methanol was rendered anhydrous by reaction with magnesium metal and collected by distillation. Methanolic HCl was prepared by bubbling HCl gas through concentrated sulfuric acid into anhydrous methanol. The molarity of the methanolic