AGING AND RAT BRAIN MUSCARINIC RECEPTORS AS MEASURED BY QUINUCLIDINYL BENZILATE BINDING

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Accepted September 4, 1979

Measurement of cholinergic muscarinic receptor binding in various rat brain areas using the ligand [3H]quinuclidinyl benzilate indicates that receptor binding is decreased in striatum and cerebellum of aged female rats (22 months old) as compared to younger rats (4 months old). Decreases were not observed in cortex, hippocampus, hypothalamus, or amygdala areas. Further examination of [3H]quinuclidinyl benzilate binding in subcellular fractions of aged and young rat cerebellum and striatum indicated a decrease in binding in the crude nuclear and crude synaptosomal fractions. Binding data indicate the observed decrease in specific ligand binding is due to a decrease in number of binding sites while receptor affinity does not appear to change.

INTRODUCTION

The muscarinic receptor for the neurotransmitter, acetylcholine, has been studied extensively in brain with respect to its ontogenesis (3, 5, 8) and distribution within various brain regions of rat (12, 23), dog (9), and monkey (25). The high concentration of muscarinic receptor found in the striatum of several species suggests an important physiological function for

1 Supported by the Research Service of the Veterans Administration and by Research Grant NS 13227 from NINCDS.
the cholinergic system within this area. The nature of the striatal cells upon which the postsynaptic muscarinic receptor are located is not known. However, the cholinergic interneurons may be under inhibitory control by dopaminergic neurons whose cell bodies originate in the substantia nigra (1). Since the catecholamine system in this area is known to change with aging (18), the present study was undertaken to determine what effects aging has on the binding of the cholinergic antagonist, \(^{3}\text{H}\)quinuclidinyl benzilate to the muscarinic receptor in the striatum and to several other brain areas.

**EXPERIMENTAL PROCEDURE**

Barrier-reared adult female Long-Evans hooded rats, ages 22 months and 4 months, were used in the study. Detailed health records were kept, randomly selected animals were autopsied trimonthly and any suspect section of the colony was sacrificed in an attempt to use only healthy animals. Animals were sacrificed by decapitation and each brain was removed, weighed, and briefly chilled on dry ice. The brain was then placed ventral side up on a multiple razor blade device and sectioned coronally. Slices were made and placed on a chilled surface and specific areas dissected out. The first slice from which hippocampus was removed included the posterior area of the brain up to the mammillary bodies (A800µ-A3000µ according to Koenig and Klippel) (11). The second slice included the area from the mammillary bodies to 2 mm anterior (A3000µ-A5000µ). The amygdala, additional hippocampus, and hypothalamus were obtained from this area. A third slice (A6000µ-A8600µ) was the source of the striatum and the cerebral cortex. Tissue was dissected, weighed, and homogenized (10 strokes) in 0.32 M sucrose. Final concentrations of whole homogenates were: cerebellum (50 mg/ml); striatum, hippocampus, and cortex (40 mg/ml); and hypothalamus and amygdala (20 mg/ml). Tissue homogenates were centrifuged at 1000 g for 10 min. The pellet containing nuclei and cellular debris was resuspended in 0.32 M sucrose. The supernatant was recentrifuged at 10,000 g for 20 min and the pellet defined as the crude membrane fraction. The supernatant was the source of enzyme in the choline acetyltransferase and cholinesterase assays. Protein determination was according to Lowry et al. (14).

An enriched nuclear fraction was prepared from rat forebrain. The tissue was homogenized in 10 volumes of 0.32 M sucrose and centrifuged at 1000 g for 10 min. The pellet was resuspended in 0.32 M sucrose and aliquots were mixed with 2.4 M sucrose (1:6) and recentrifuged at 25000 g for 1 hr. The pellet was resuspended in 0.32 M sucrose. Examination after staining (1.6% Geimsa in alcohol) showed an enriched nuclei fraction in the pellet and relatively few nuclei in the supernatant fraction which contained mostly membranous material.

Muscarinic receptor binding was carried out according to Yamamura and Snyder (23). Briefly, tissue homogenate containing 20–100 µg of protein in 0.5 ml of 0.05 M sodium-potassium phosphate buffer, pH 7.4, was incubated with 1.5 nM \(^{3}\text{H}\)quinuclidinyl benzylate, \(^{3}\text{H}\text{QNB}\) at 24°C. A duplicate set of tissue samples containing additionally 0.1 µM atropine sulfate were likewise incubated. After 60 min, 3 ml of iced buffer was added to the incubation tube and the contents were collected on Whatman glass fiber filter disks (GFC). After rinsing 3 times with 3 ml of iced buffer, each filter disk was placed in a vial along with 10 ml scintillation fluoros and \(^{3}\text{H}\text{QNB}\) bound to protein measured by liquid scintillation counting. The amount of specifically bound ligand was designated as the dif-