ALTERATIONS IN ATROPINE SITES OF THE BRAIN OF RATS AS A FUNCTION OF AGE

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SUMMARY - Binding of (^{3}H)-atropine to synaptosomal fractions prepared from cerebral and cerebellar cortices of young, adult and old male and female rats were studied. Picomoles of labelled atropine bound/mg protein was highest in the cerebral cortex of young rats and decreased with increasing age in both sexes, whereas in cerebellar cortex the peak binding was in adult rats. Acetylcholinesterase activity ofthe same fractions showed corresponding changes with age.

Several functions like memory, learning and behavioral adjustments decrease with increasing age (1). These changes may be due to alterations in biochemical responsiveness of neurones to various stimuli including hormones, neurohumors and neurotransmitters. One of such changes may be the binding of these molecules to their respective receptors. Roth (2) reported that the specific glucocorticoid binding sites in the brain decrease in old rats. Studies from this laboratory have shown that the level of 17β-estradiol binding protein of the cytosol of the brain of the rat decreases in old age (3), and this directly corresponds to the changes in the induction patterns of acetylcholinesterase (AChE) by this hormone (4). Walker and Boas-Walker (5) demonstrated that the sensitivity of adenyl cyclase to neurohumors is reduced in the caudate, cerebellum, cortex and hippocampus of senescent rats. Acetylcholine (ACh), the cholinergic neurotransmitter, exerts its effect by binding to one of the two specific receptors, muscarinic or nicotinic, depending upon the physiological condition. These

receptors are located on the post-synaptic membrane. A number of studies favour the presence of both muscarinic (6-9) and nicotinic (10-12) types of receptors of ACh in the brain. The present study was undertaken to investigate the binding parameters of atropine, an inhibitor of muscarinic receptors, to synaptosomal fractions prepared from cerebral and cerebellar cortices of the brain of rats of three different ages.

MATERIALS AND METHODS - (^{3}H)-Atropine (350 mCi/mmole) was purchased from The Radiochemical Centre, Amersham, and atropine was obtained from Sigma Chemical Co., U.S.A. Young (8-), adult (40-) and old (80-week) male and female albino rats of Wistar strain were used. They were kept at 24 ± 2°C under an artificial illumination programmed to provide 12 hr light starting from 7.00 A.M. followed by a 12 hr dark period. They were fed gram (Cicer arietinum) and a freshly prepared diet containing flour and vitaminized powdered milk (4:1) in water. Tap water was supplied ad lib. 4-5 rats from both sexes of each age group were used.

Synaptosomal fractions were prepared according to Whittaker (13). Rats were killed at a fixed time of the day by cervical dislocation and cerebral and cerebellar cortices were homogenized in 10 vol. of ice-cold 0.32 M sucrose. The homogenate was centrifuged at 1,000 g for 10 min. at 0°C in a refrigerated centrifuge (IEC, PR-6 model). The supernatant was then centrifuged at 10,000 g and the resulting synaptosomal pellet was resuspended in 0.32 M sucrose and the protein was adjusted to approximately 1 mg/ml. Aliquotes from this were used for muscarinic binding studies.

Muscarinic binding sites were quantitated according to Bartfai et al (7). Samples containing 0.2-1.0 mg/ml protein were incubated

for 1 hr at 37°C with 3.0 ml Ringer solution (3.0 mM Na_2HPO_4, 2.1 mM KCl, 1.8 mM $CaCl_2$, 116 mM NaCl, pH adjusted to 7.2) containing 10^{-7}M (^{3}H)-atropine (96,000 cpm). The pre-soaked Metricel membrane filter (pore size, 0.45 μm) was washed with 15 ml of ice-cold distilled water after which the sample was rapidly filtered under suction. The filter was rinsed and dried. It was then placed in 10 ml toluene phosphor in a standard scintillation vial and counted in a Beckman LS-100C Liquid Scintillation Spectrometer at 39% efficiency in the tritium channel. Each determination was made in triplicate together with duplicate samples containing unlabelled atropine (1 mM) to determine the non-specific binding. Specific binding was obtained by substracting the amount not displaced by higher concentration of unlabelled atropine from the total bound radioactivities. The atropine binding was measured by equilibrium dialysis (14) to check the efficiency of the filter assay.

Protein was determined according to Sutherland et al (15) and acetylcholinesterase was assayed as described by Ellman et al (16).

RESULTS - The binding of (^{3}H)-atropine is maximum in the synaptosomal fraction prepared from the cerebral cortex of young rats, irrespective of sex (Fig. 1). Picomoles of atropine bound/mg protein gradually declines as a function of age and is lowest in old rats. However, in the cerebellum, the peak binding of (^{3}H)-atropine/mg protein is observed in adult rats and decreases thereafter. Similar results are obtained for both the sexes.

Correlative changes are observed when the amount of labelled atropine binding to synaptosomal preparations from cerebral and cerebellar cortices of rats of different ages are compared with AChE activity of the same fractions. The enzyme activity at various

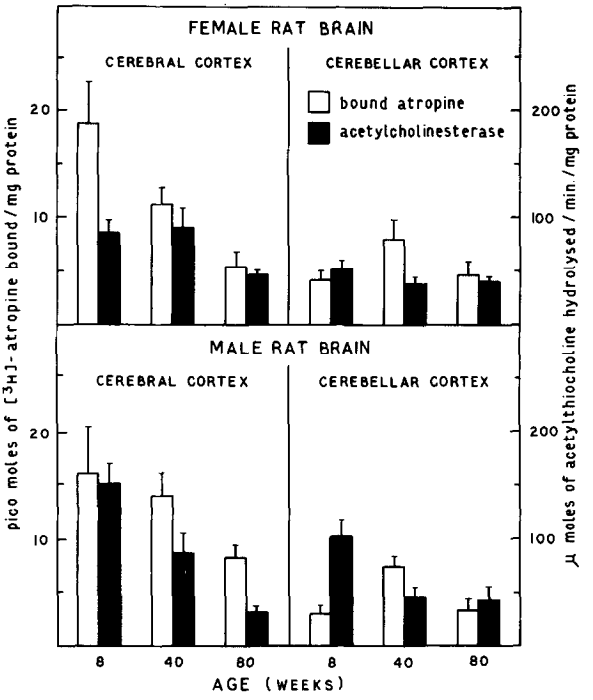

Fig. 1. Atropine binding and acetylthiocholine hydrolysis as functions of age.

ages follows the same pattern as that of atropine binding. AChE level is high in cerebral cortices of young and adult rats of both the sexes. The activity of AChE is very low in old rats. In adult male rats it is significantly lower than in young rats, though (^{3}H)-atropine binding is similar in both the sexes with the peak in the adult.

DISCUSSION - Impairment of major brain functions like learning, memory and adaptive behavior are associated with aging (1). The functional efficiency of the brain depends on the proper working of the neurotransmittor system. The binding of ACh to its specific receptor changes the permeability of the synaptic membrane to Na ion which triggers the nerve impulse. It has been shown that the activities

of enzymes related to ACh metabolism, cholinesterase (17, 18) and AChE (4), decrease with age. The activity of choline acetyltransferase, the synthetic enzyme for ACh, also decreases in old rats (M.S.K. and T.C.J. unpublished data).

The highest level of atropine binding sites/mg protein in the young may be correlated with the high level of AChE at this age. This may be associated with learning and memory (19). The gradual decline in the number of binding sites after attainment of maturity may be due to a loss of neurones from the brain that occurs with increasing age (20) followed by re-occupation of the space by glial cells which may be devoid of ACh receptors. It is also possible that the decrease in atropine binding may be due to synaptic degeneration in old age. The levels of AChE, and atropine binding sites, in general, follow a similar pattern in the cerebellum. The few discrepancies observed may be due to measurement of only a fraction of the total ACh receptors.

Studies from this laboratory (21, 22) show that programmed changes in the activities of genes begin after fertilization and continue throughout the life-span (23, 24). The decrease in the levels of atropine binding macromolecules in old rats may be due to a gradual decrease in the synthesis of this protein after maturity according to this programme.

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