# INDUCTION OF ACETYLCHOLINESTERASE BY 17-β ESTRADIOL IN THE BRAIN OF RATS OF VARIOUS AGES[1]

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SUMMARY - The induction of acetylcholinesterase (AChE) was studied in the cerebral hemisphere and cerebellum of immature (9-), adult (29-) and old (65-week) female rats. The specific activity of AChE of the cerebral hemisphere of normal rats is highest at 9 weeks and decreases thereafter. There is no such change in the cerebellum. Ovariectomy decreases its activity in the cerebral hemisphere of adult, and cerebellum of immature and adult rats, but not of old rats. Administration of estradiol to ovariectomized rats increases the activity in the cerebral hemisphere and cerebellum of immature and adult rats but not of old rats. The magnitude of stimulation, which is actinomycin D-sensitive, is highest in immature rats.

INTRODUCTION - Alterations in qualitative and quantitative nature of enzymes may contribute to aging. The decrease or increase in the activity of enzymes may be due to changes in the template activity of corresponding genes. The induction of cytoplasmic(c-) and mitochondrial(m-) malate dehydrogenase (MDH), and glutamine synthetase (GS) of the liver by cortisone has been studied as a function of age of the rat in this labolatory (1, 2). The hormone induces mMDH of young rats but not of old rats, whereas it induces cMDH and GS throughout the life span, though decreasing with age. The induction of these enzymes is actinomycin D-sensitive.

Acetylcholinesterase (AChE; E.C. 3.1.1.7.) is of vital importance for nerve function. Gonadal hormones which are

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important for behaviour may exert their influence through the brain. This paper contains the first report that (a) a brain enzyme (AChE) is induced by a sex hormone (17-β estradiol), (b) there is a difference in the induction of the enzyme in the cerebral hemisphere and cerebellum and (c) this induction in the rat is age-dependent.

MATERIALS AND METHODS - Immature (9-), adult (29-) and old (65-week) female albino rats of Wistar strain were used. They were kept at 24 ± 2°C under an artificial illumination programme to provide 12 hr light starting from 7.00 A.M. followed by a dark period. They were fed standard Anidiet 'A' (Chelsea Chemical Laboratories, Poona), gram (Cicer arietinum), and a diet containing powdered milk and flour (1:4) prepared daily.

Female rats of each of the three age groups were divided into four groups, having 4-6 animals. Group I consisted of intact rats which were given 0.9% NaCl i.p. Group II, III and IV rats were bilaterally ovariectomized and were kept on tap water and standard diet for 21 days. On the 22nd day, group II rats were given 0.9% NaCl (1.0 ml), group III rats were injected 17-β estradiol (10 µg/100 g body wt. in 1.0 ml of 0.9% NaCl) and group IV rats were given actinomycin D (10 µg/100 g body wt. in 1.0 ml of 0.9% NaCl) prior to estradiol injection. The rats were killed 4 hr after the estradiol injection. Group II rats served as control for the induction of AChE by estradiol.

The cerebral hemisphere and cerebellum were dissected out and chilled. A 2% homogenate (w/v) was prepared in 0.1 M ice-cold phosphate buffer (pH, 8.0). AChE was assayed spectrophotometrically (3). Protein was estimated (4), and the enzyme activity was expressed as specific activity (units/mg protein).

Each set of data was collected from 4-6 rats of a specific age group and was statistically analysed. 5% or lower values of $p$ were taken as significant.

RESULTS AND DISCUSSION - The changes in the levels of enzymes (5), regulation by effectors (6), and inducibility by certain hormones (1,2,7,8) are influenced by age. Our data show that the activity of AChE of the cerebral hemisphere is highest in immature rats (9 week), decreases by 50% by 29 weeks and further decreases by 65 weeks of age. However, the activity of AChE in the cerebellum does not show any significant change with increasing age starting from 9 weeks. The activity in the cerebral hemisphere of immature rat is approximately 2.6-fold higher than that of cerebellum (Table I). Since 9-week old rat is in the learning phase, a higher activity of AChE at this age may facilitate the learning process. The decrease in the enzyme activity in adult and old rats may be due to a loss of nerve cells. Another reason may be that the rate of synthesis of the enzyme decreases after the growth period. This is supported by the finding that polyribosome content of the brain decreases in old age (9). The activity of AChE of the cerebellum does not change with age. This shows the differential metabolic activities of different parts of the brain as a function of age.

The levels of several enzymes are known to decrease following ovariectomy suggesting a possible role of estrogen in the regulation of these enzymes. The present study shows that ovariectomy lowers the activity of AChE in the cerebral hemisphere of the adult, and in the cerebellum of immature and adult rats. There is a small increase in its activity following ovariectomy in the cerebral hemisphere of immature

rat which may be due to a compensatory hypertrophy of the adrenal which is known to produce small amounts of estrogen. However, ovariectomy has no effect in the old. This is supported by the finding that ovariectomy does not alter the level of enzymes in the old (10).

Administration of estradiol to ovariectomized rats increases the activity of AChE of the cerebral hemisphere and cerebellum of immature and adult rats, but has no effect in the old. Administration of actinomycin D significantly lowers the activity in both the organs of the immature, but has no effect in the cerebral hemisphere of the adult. Therefore, estradiol-mediated induction may be due to an increase in the transcription of mRNA which increases the synthesis of the enzyme.

Our studies show that the enzyme is maximally induced in both the organs of immature rats and the percentage of stimulation is lower in the adult. The hormone does not induce the enzyme in the old. This may be due to a differential responsiveness of the gene for AChE to the hormone at different ages. The decrease in estradiol-mediated stimulatory effect in the adult and the absence of this effect in the old suggest that the level of estrogen receptor decreases with age. Alternatively, the appearance of a repressor of the gene for AChE in old age may prevent the binding of estrogen-receptor complex to the gene and thus prevent induction. The level of estrogen in women is known to decrease in old age. Whether this decrease affects the level of AChE of the brain and thereby the behaviour pattern of the female is not known. These findings are consistent with the gene regulation theory proposed by Kanungo (5) according to which the

TABLE I

EFFECT OF OVARIECTOMY (O♂), ESTRADIOL (E) AND ACTINOMYCIN D (A) ON SPECIFIC ACTIVITY OF CEREBRAL HEMISPHERE (CH) AND CEREBELLUM (CB) OF RATS OF DIFFERENT AGES

| Organ | Treatment | 9 week Mean ± S.D. | p | 29 week Mean ± S.D. | p | 65 week Mean ± S.D. | p |
|---|---|---|---|---|---|---|---|
| CH | N | 126.64 ± 4.46 | < 0.01 | 62.98 ± 2.36 | < 0.01 | 44.95 ± 4.53 | > 0.10 |
| | O♂ | 162.26 ± 4.81 (+34.90%) | < 0.01 | 46.46 ± 3.12 (-25.80%) | < 0.01 | 47.33 ± 1.23 (NE) | > 0.10 |
| | O♂ + E | 319.08 ± 34.21 (+113.10%) | < 0.01 | 79.10 ± 5.04 (70.30%) | > 0.10 | 45.09 ± 2.18 (NE) | < 0.01 |
| | O♂ + A + E | 158.06 ± 6.06 (-54.30%) | | 92.75 ± 11.56 (NE) | | 37.96 ± 17.40 (-17.40%) | |
| CB | N | 48.48 ± 4.64 | < 0.01 | 44.59 ± 2.43 | < 0.01 | 42.35 ± 2.43 | > 0.05 |
| | O♂ | 31.87 ± 0.73 (-34.60%) | < 0.01 | 37.59 ± 0.55 (-14.40%) | < 0.01 | 36.07 ± 1.52 (NE) | > 0.10 |
| | O♂ + E | 83.68 ± 3.99 (+166.30%) | < 0.01 | 56.41 ± 2.04 (+51.20%) | < 0.01 | 32.17 ± 1.33 (NE) | > 0.10 |
| | O♂ + A + E | 35.61 ± 2.54 (-57.30%) | | 46.70 ± 2.72 (-17.10%) | | 33.37 ± 0.67 (NE) | |

+, stimulation; -, inhibition; NE, no effect; N, normal. Mean values from 4-6 rats of each age are given.

changes in the level of enzymes seen during aging may be due to alterations in the template activity of corresponding genes that may be brought about by various effectors or modulators produced during growth.

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