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MODULATION OF ACETYLATION OF HISTONES AND TRANSCRIPTION OF CHROMATIN BY BUTYRIC ACID AND 17β-ESTRADIOL IN THE BRAIN OF RATS OF VARIOUS AGES

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ABSTRACT - The effects of butyric acid and 17β-estradiol on *in vitro* acetylation of histones and transcription of nuclei of cerebral cortex were studied in female rats of various ages. Incorporation of UMP into RNA of acetylated nuclei is higher than that of normal. The template activity of nuclear DNA of normal and acetylated nuclei decreases with increasing age of the rat. The decrease is correlated with the decline in acetylation of histones. Both butyric acid and estradiol stimulate transcription in young and adult rats. Butyric acid, unlike estradiol, shows marked stimulatory effect in the young. However, these modulators have no significant effect in old age. Such age-related transcriptional modifications of the chromatin of rat brain brought about by different effectors may be responsible for a gradual decline in the functional activity of the organism and lead to aging.

INTRODUCTION - Acetylation of histones has been correlated with gene activation (1). Structural modifications of nucleosomal histones by acetylation is reported to determine the number of initiation sites available on chromatin for binding to RNA polymerase (2). We have recently shown that 17β-estradiol stimulates acetylation of nucleosomal histones of the brain of rats, and that this effect decreases with age (3). Na-butyrate has been shown to increase acetylation of nucleosomal histones (4) by inhibiting histone deacetylase (5). In the present investigation we have attempted to answer two questions: (a) whether increase in acetylation of histones by Na-butyrate is age-dependent, and (b) whether acetylation of histones by Na-butyrate and 17β-estradiol has any age-related effect on transcription in the brain of rats.

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EXPERIMENTAL - Young (4-), adult (15-) and old (110-week) female albino rats of Wistar strain maintained in the rat colony were used. The cerebral cortex was excised immediately after decapitation, and cut into 0.4 mm slices. Acetylation of chromosomal proteins was carried out by incubating 1.0 g of the sliced tissue with ^{14}C-Na-acetate (sp. act. 41.8 mCi/mmole; Bhabha Atomic Research Centre, Bombay) for 1.0 h (3,6). The effects of Na-butyrate and 17β-estradiol were studied by adding 5.0 mmole and 1.0 μmole of the effectors, respectively, into the incubation medium 30 min. prior to the addition of ^{14}C-acetate. The slices were washed in ice-cold buffer after 1.0 h of incubation and homogenised in 10 vol. of 0.32 M sucrose-3 mM $MgCl_2$. The nuclei were isolated by sucrose gradient (3,7).

The template activity of chromatin for RNA synthesis was studied (8) by placing the nuclei in a reaction mixture (0.25 ml final vol.) consisting of 20 mM Tris-HCl buffer, pH 8.0; 120 mM KCl; 0.1 mM EDTA; 2.5 mM $MnCl_2$; 0.1 mM dithiothreitol; 0.8 mM each of ATP, GTP and CTP; 0.2 mM (^{3}H) UTP (sp. act. 50 μCi/mmole; Radiochemical Centre, Amersham). The concentration of nuclei in each assay was 50 μg with respect to DNA. The assay mixture was incubated at 37°C for 30 min. The reaction was stopped by adding 5 ml of 10% TCA/1% Na-pyrophosphate solution. The reaction in the control tubes was stopped at zero-time. The acid insoluble material was filtered by glass filter discs (presoaked in 10% TCA). The radioactivity in the filter discs was counted in toluene-scintillator using a Beckman LS-100C scintillation counter. The incorporation of (^{3}H) UMP into RNA was determined by dividing the total counts in RNA (minus the counts in the control) by specific activity of the labelled UTP, and expressed as nmoles (^{3}H) UMP/mg DNA.

Histones were isolated from nuclei (9) and their radioactivity was estimated using triton-toluene scintillator. Then the specific activity (CPM/μg histone) of acetylated histones was determined.

RESULTS AND DISCUSSION - Table 1 shows that acetylation of histones decreases progressively with age as reported earlier by us (3). Acetylation at 110 weeks is only 50% of that at 4 weeks. Butyric acid increases acetylation of histones significantly both in the young and the adult, but has no effect in the old. Estradiol increases acetylation only in the young.

Table 2 shows that there is a gradual decrease in *in vitro* RNA synthesis with increasing age. Transcription is greatly increased after acetylation of histones in the immature. This effect is lower in the adult, and is not observed in the old. When butyrate is present in addition to acetate, the degree of transcription is even greater both in the immature and the adult. Butyrate has no effect in the old. Estradiol, like butyrate, potentiates trans-

Table 1. Effects of butyric acid (BA) and estradiol (Est) on specific activity (CPM/µg protein) of acetylation of histones of the cerebral cortex of female rats of various ages.

Age (weeks)	Slice + Ac^-	Slice + BA + Ac^-	Slice + Est + Ac^-
4	0.69	0.84	0.79
32	0.43	0.67	0.41
110	0.37	0.38	0.38

Data are averages obtained from 4-5 rats of each age.

Table 2. Incorporation of (^3H) UMP (nmoles/mg DNA) into RNA of acetylated nuclei of cerebral cortex of female rats of different ages and its modulation by butyric acid (BA) and estradiol (Est).

Age (weeks)	Slice	Slice + Ac^-	Slice + BA + Ac^-	Slice + Est + Ac^-
4	8.86	17.29	22.36	20.68
32	7.40	9.30	10.27	14.78
110	6.14	6.51	6.98	6.16

Data are averages obtained from 4-5 rats of each age.

cription both in the immature and the adult, but has no effect in the old.

The precise mechanism whereby transcription in eukaryotes is regulated is not known. It appears reasonable that covalent modifications of chromosomal proteins such as phosphorylation, acetylation, methylation and ADP- ribosylation may alter their interaction with DNA, and thereby modulate the expression of specific genes (1,10). Acetylation of histones, especially that of nucleosome, has been shown to stimulate transcription (11, 12). This is believed to be due to conformational changes in chromatin that follows ace-

tylation (13,14). Na-butyrate causes hyperacetylation of H3 and H4 histones (4,15,16) which is due to inhibition of histone deacetylase (5,17). Acetylation of H3 and H4 is reported to inhibit DNA synthesis (18). Unlike phosphorylation which occurs largely in H1 histones and in dividing cells, acetylation occurs mainly in H3 and H4 histones, and in non-dividing but metabolically active cells. Thus acetylation of histones appears to be essential for transcription.

Our studies show that tissue slices are good model systems to study age-related changes in chromatin function. The age-dependent decrease in acetylation of histones of the brain may be due to one or more of the following causes: a decrease in permeability of acetate through the cell membrane, a decrease in the level of acetyltransferase, or changes in chromatin conformation such that lysyl residues are not available for acetylation. As shown for HeLa cells (4,5,15,16) Na-butyrate increases acetylation of histones of thebrain of immature and adult rats. Estradiol also increases acetylation in the immature, but its mechanism of action is not known. It has been shown that estradiol induces acetylcholinesterase (AChE) in the brain of rats (19,20) which is mediated through receptors (21). Furthermore, the induction of AChE is impaired in old age which appears to be due to depletion of specific estradiol-receptors in the brain (22). Since in our study estradiol stimulates not only acetylation, but also transcription, it is suggested that estradiol may stimulate transcription of specific genes by increasing acetylation of nucleosomal histones.

In addition, these studies show that not only normal acetylation decreases with age, but also such effectors as Na-butyrate and estradiol fail to stimulate acetylation of histones in old age. Consequently, the degree of transcription is also decreased. Thus

there is direct correlation between acetylation of histones and transcription which is consistent with the earlier findings (11,12). The decrease in both acetylation of histones and transcription of chromatin of the brain with increasing age of the rat may be due to conformational changes in the chromatin of the brain cells. It is possible also that a decrease in acetyltransferase and RNA polymerase may contribute to such alterations. These studies further support the view that sequential changes in the expression of genes may be brought about by various effectors including hormones and lead to senescence (23).

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