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CHROMATIN FUNCTION IN DEVELOPING BRAIN: ACETYLATION OF CHROMOSOMAL PROTEINS AND RNA SYNTHESIS

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SUMMARY - In vitro acetylation of chromosomal proteins and RNA synthesis, and their modulation by spermine were studied using slices of cerebral cortex of 3 - 30 day old developing male rats. The degree of acetylation of both histones and nonhistone chromosomal (NHC) proteins, and RNA synthesis are high at day 3 and decrease rapidly as development progresses. Spermine stimulates acetylation of histones and NHC proteins. It also stimulates incorporation of ^{3}H-Uridine both into nuclear and cytoplasmic RNAs. These stimulatory effects decrease rapidly up to day 9 and then decrease more slowly. Such chemical and functional changes in chromatin may be necessary for the terminal differentiation of neurons, and contribute to differential gene expression during development.

INTRODUCTION - In eukaryotes, DNA is complexed with histones and nonhistone chromosomal (NHC) proteins. Both histones and NHC proteins undergo several post-synthetic modifications like phosphorylation, acetylation, methylation and ADP-ribosylation which are believed to play important roles in the structure and function of chromatin. Histone acetylation may be involved in transcriptional activity (1,2). Histones are bound to DNA by electrostatic interactions involving positively charged residues in the NH_2-terminal end of histones and the negatively charged phosphate backbone of DNA (3,4), in addition to hydrophobic interactions (5). Many of the lysine residues in the NH_2-terminal region of histone H_3 and H4 are subject to highly active post-synthetic acetylation and deacetylation (6,7). Acetylation

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partially neutralizes the basic charges of histones, causing localized displacement from DNA, and possibly conformational changes in chromatin leading to stimulation of transcription (8). NHC proteins are highly heterogeneous and tissue- and species-specific (9). Little is known about their acetylation except that a small group known as high mobility group proteins of calf thymus and duck erythrocytes are acetylated (10).

The present in vitro studies examine the changes in acetylation of both histones and NHC proteins, and RNA synthesis in the cerebral cortex during early post-natal development of the rat. Since polyamines have been implicated in cell multiplication and growth (11), the effect of spermine on acetylation of chromosomal proteins and RNA synthesis were also studied. This may give some insight into the structural changes that may occur in the chromatin as a proliferating neuron enters the nonproliferative state during development.

MATERIALS AND METHODS - Three-, 9-, 14-, 18-, 23- and 30-day old male rats were killed by cervical dislocation, and cerebral cortex was excised and immediately cut into slices of approximately 0.4 mm thickness. In vitro acetylation (12) of histones and NHC proteins was studied by incubating 1.0 g of sliced tissue in flasks containing 4.0 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, bubbled with a mixture of 95% O_2 and 5% CO_2. Cycloheximide (2 x 10^{-4}M) was added to each flask to inhibit protein synthesis. Spermine (10^{-5}M) was added to the experimental flasks. Control and experimental flasks were incubated in duplicate in a water bath at 37°C for 20 min with constant shaking. Then 0.1 mCi of (U-^{14}C) Na-acetate (sp. act. 49.3 mCi/mmole; Bhabha Atomic Research Centre (BARC) Bombay) was added to each flask. RNA synthesis was studied by adding 0.1 mCi of ^{3}H-Uridine (sp. act. 10,900 mCi/mmole, BARC) to the incubation medium 20 min after the addition of labelled acetate. Incubation was continued for 50 min after which the slices were taken out and washed thrice with cold KRB-buffer. Pilot experiments showed that oxygen consumption by the slices was linear up to 90 min.

Chromatin was purified from the tissue according to Bonner et al (13). Phenylmethylsulfonylfluoride (0.5 mM) was used in all buffers to inhibit protease activity. Chromatin was sheared and fractionated to extract histones (14). The dehistonized chromatin was treated with bovine pancreatic DNase I (Sigma Chemical Co.,

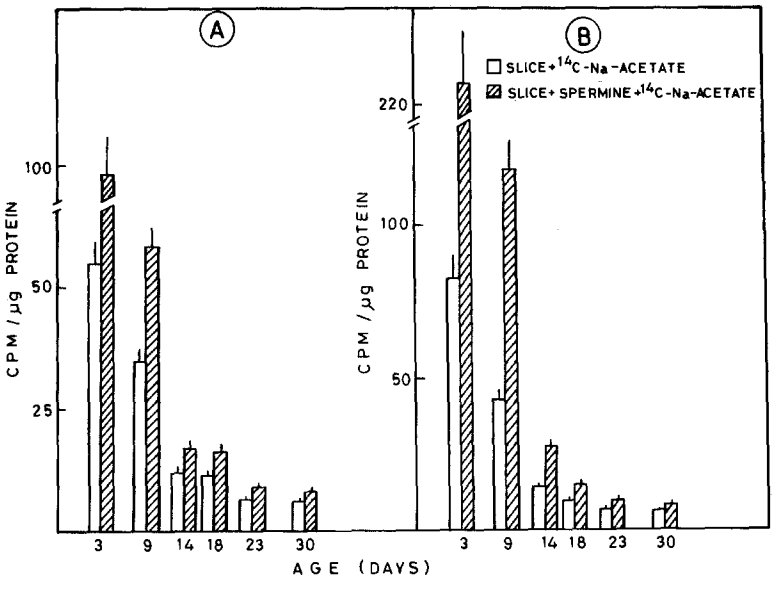

Fig. 1. Effect of spermine (10^{-5}M) on in vitro acetylation of histones (A), and NHC proteins (B) of cerebral cortex of developing male rats. Data were collected from 6-8 rats of each age.

U.S.A.) to separate DNA from NHC proteins (15). Radioactivity in each fraction was estimated using triton-toluene scintillator (16) in a Beckman LS-100C scintillation counter. The contents of histones and NHC proteins were determined (17).

The incorporation of ^{3}H-Uridine into nuclear and cytoplasmic RNAs was determined by measuring the radioactivity in each fraction. The RNA content of both the fractions was determined (18).

RESULTS AND DISCUSSION - Fig. 1. shows that acetylation of both histones and NHC proteins decreases rapidly till about day 14 and then plateaus. The low degree of acetylation of chromosomal proteins after 14 days of development may be due to one or more of the following factors: (a) Cessation of neuronal division that occurs around this age, (b) decrease in the activity of acetyltransferase, and (c) the non-availability of lysyl residues for acetylation due to conformational changes in the chromatin as the cells enter non-dividing state during development.

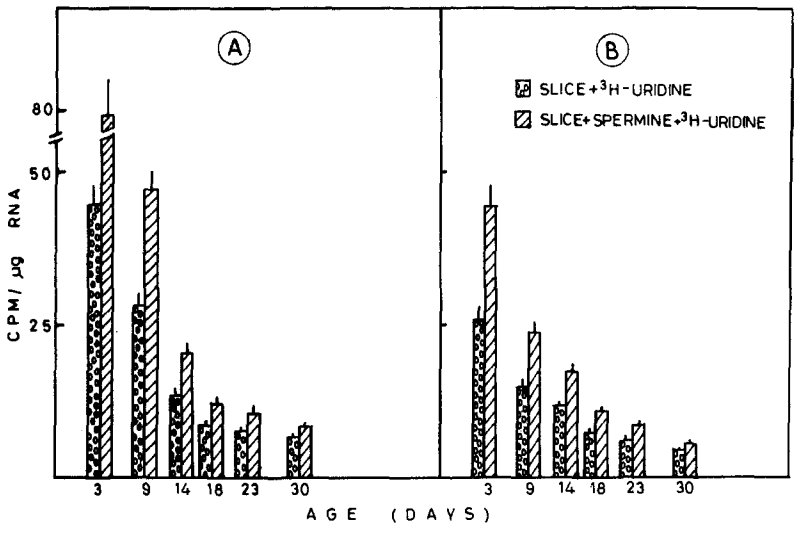

Fig. 2. Effect of spermine (10^{-5}M) on in vitro incorporation of ^{3}H-Uridine into nuclear (A), and cytoplasmic RNA (B) of cerebral cortex of developing male rats. Data were collected from 6-8 rats of each age.

Spermine stimulates acetylation of both histones and NHC proteins, and the degree of stimulation is greater for NHC proteins (Fig. 1). The stimulatory effect decreases in both the cases after about 14 days of development. Spermine is reported to bind directly to DNA (19), and may thereby expose more sites in chromosomal proteins for acetylation. The decrease in its stimulatory effect after day 14 may be due to conformational changes in the chromatin that occur during development. Histone acetylation is reported to occur prior to an increase in RNA synthesis (16). Also, stimulation of acetylation by polyamines is believed to occur during the developing phase (20).

Fig. 2. shows that the radioactivity in both the nuclear and cytoplasmic RNAs of the cerebral cortex decreases rapidly during early development. The incorporation of ^{3}H-Uridine into nuclear RNA is more than that of the cytoplasmic RNA. This may be because the fraction of RNA entering the cytoplasm from the

nucleus is lower due to processing of HnRNA in the nucleus. Polyamines are known to stimulate transcription (21,22). Our data show that spermine stimulates incorporation of ^{3}H-Uridine into both nuclear and cytoplasmic RNAs. The stimulatory effect decreases in both the cases during development. This may be due to changes occurring in the chromatin during this period. Acetylation of lysyl residues of chromosomal proteins is reported to alter the conformation of chromatin and stimulation of RNA synthesis (23). Hence the decrease in the acetylation of chromosomal proteins, particularly of histones during development observed by us, indicates that histones may get more tightly bound to DNA as development progresses (24), and this may be responsible for the decline in RNA synthesis (25, 26). The stimulatory effects of spermine on acetylation of chromosomal proteins, and on transcription in early developmental period reported here show that polyamines may be involved in differential gene expression. Such changes in the modification of chromosomal proteins and their modulation by effectors like spermine, whose levels also change during development, may be responsible for differential gene expression and lead to cellular differentiation, development, and senescence (27, 28).

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