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PHOSPHORYLATION AND ACETYLATION OF NONHISTONE CHROMOSOMAL PROTEINS OF THE BRAIN OF RATS OF VARIOUS AGES AND THEIR MODULATION BY CALCIUM AND ESTRADIOL

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ABSTRACT - In vitro phosphorylation and acetylation of nonhistone chromosomal (NHC) proteins and their modulation by Ca^{++} and estradiol were studied by incubating slices of cerebral cortex of 2-, 15- and 84-week female rats with ^{32}Pi and ^{14}C-Na-acetate. Phosphorylation pattern of NHC proteins is unique for each age. Ca^{++} and estradiol stimulate phosphorylation of different NHC proteins which is also age-specific. Acetylation of NHC proteins decreases precipitously with age. No unique NHC protein is acetylated preferentially at any age, nor does Ca^{++} stimulate acetylation. Estradiol, however, stimulates acetylation of a few NHC proteins. It is suggested that phosphorylation of NHC proteins and its modulation by effectors may be more important for gene expression than their acetylation.

INTRODUCTION

Non-histone chromosomal (NHC) proteins are large in number, highly heterogenous, acidic, and tissue-and species-specific (1). They are implicated in gene expression in general (2-5) and in the control of transcription in particular (6,7). NHC proteins also undergo covalent modifications like phosphorylation and acetylation (1). Phosphorylated NHC proteins have been implicated in the expression of specific genes (5,8). Phosphorylation of NHC proteins stimulates transcription and dephosphorylation inhibits it (9). Steroid hormones stimulate phosphorylation of NHC proteins (10). Information about acetylation of NHC proteins is lacking. We show here that NHC proteins of the brain of rats undergo differential phosphorylation and acetylation, and that these modifications are modulated by calcium and estradiol. Furthermore, these modifications are age-dependent.

EXPERIMENTAL

Immature (2-week), adult (15-week) and old (84-week) female rats of Wistar strain were used. Cerebral cortices of the rats were excised and immediately

Abbreviations: NHC proteins, non-histone chromosomal proteins; SDS, sodium dodecyl sulfate.

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frozen and cut into 0.4 mm thick slices. In vitro phosphorylation and acetylation of NHC proteins were studied (11) by incubating 1.0 g of the sliced tissue in flasks containing 4.0 ml Krebs-Ringer bicarbonate buffer, pH 7.4. Phosphate free buffer was used for the study of phosphorylation. 17β-estradiol (1.0 μmole) or calcium gluconate (5.0 μmole) was added to experimental flasks. Cycloheximide (2×10^{-4}M) was also added to inhibit protein synthesis. The control flasks did not contain 17β-estradiol or calcium gluconate. The control and experimental flasks were set up in duplicate in a water bath at 37°C and shaken for 30 min. Then 0.1 mCi of ^{32}P-orthophosphate and 0.1 mCi of (U-^{14}C) sodium acetate (Bhabha Atomic Research Centre, Bombay) were added to the flasks set up for phosphorylation and acetylation, respectively. The shaking was then continued for 60 min.

The slices were then taken out and washed thrice in cold buffer. Chromatin was purified from the tissue (12) and NHC proteins were isolated from the chromatin (13) after extraction of histones. 50 μg of NHC protein was loaded on sodium dodecyl sulfate-polyacrylamide gels for electrophoresis for 4.5 hr. at 80 V. The gels were stained and cut into 2 mm slices by a Bio-Rad Model 190 electrophoresis gel slicer. Radioactivity in each slice was counted in an LS-100C Beckman scintillation counter after digestion of the slices in 0.2 ml of 30% H_2O_2 (v/v).

RESULTS AND DISCUSSION

SDS-polyacrylamide gel electrophoresis of NHC proteins of the brain of rats shows that a few unique bands are present in each age (Fig. 1). Fig. 2a, b show that the phosphorylation pattern of NHC protein fractions is also unique for each age. In the immature, the 13th band is significantly phosphorylated. In the adult, the 15th and 27th bands are also phosphorylated. This differential phosphorylation of specific NHC protein fractions is not seen in old age. Calcium stimulates phosphorylation of a few specific NHC protein fractions in each age, that is, the 18th in the immature, 9th and 22nd in the adult, and 22nd and 24th in the old (Fig. 2a). 17β-Estradiol stimulates phosphorylation of 18th and 21st bands, but inhibits that of 13th in the immature (Fig. 2b). In the adult, the 21st band is stimulated, but the 15th is inhibited. There seems to be little effect of estradiol in old age.

The decrease in acetylation of NHC proteins in the adult appears to be mainly due to a decrease in the acetylation of high M.W. fractions (Fig. 3). In the old, all NHC protein fractions are acetylated to a lesser degree. No unique fraction of NHC protein is acetylated at any age. Calcium appears to inhibit acetylation of high M.W. NHC proteins in the adult. Estradiol stimulates acetylation of a few unique NHC protein fractions in the adult (bands 14 and 23), but inhibits that of high M.W. NHC proteins. In the old acetylation of 14th, 21st and 22nd bands is stimulated.

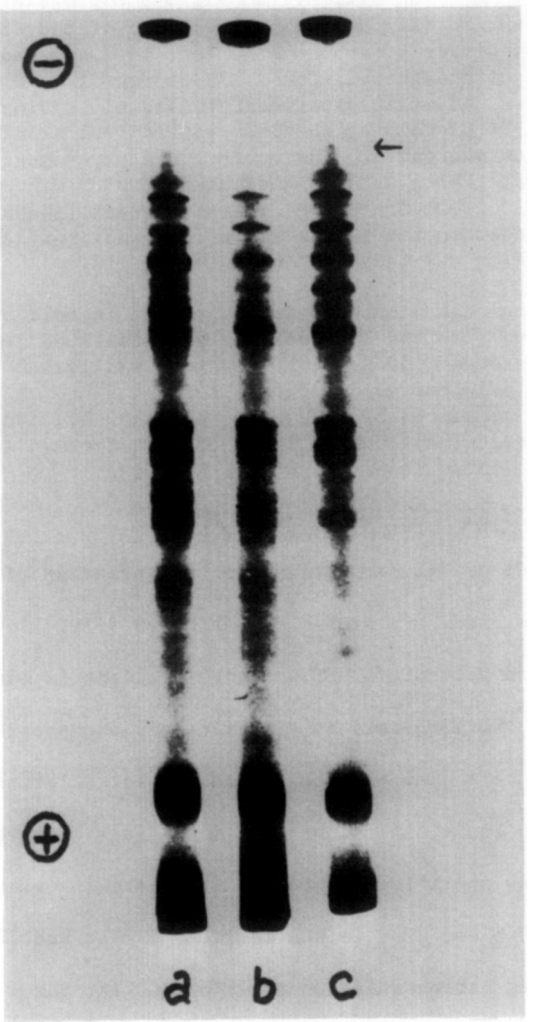

Fig. 1 SDS-polyacrylamide gel electrophoresis of NHC proteins of cerebral cortex of (a) 2-week, (b) 15-week and (c) 84-week female rats. The gel was cut into 30, 2mm slices beginning from the top stained region (arrow) and numbered 1-30.

The NHC proteins have regulatory role in gene expression (2-7). However, the role of individual NHC proteins is not known. The electrophoretic patterns of NHC proteins of the brain are unique for each age studied, and a few bands disappear with increasing age and simultaneously a few new bands appear. This suggests that there may be a concomitant alteration in gene expression. Such alterations may be mediated or accentuated by endogenous factors like calcium and estradiol. Calcium is necessary for the catalytic activity of protein kinases that phosphorylate certain

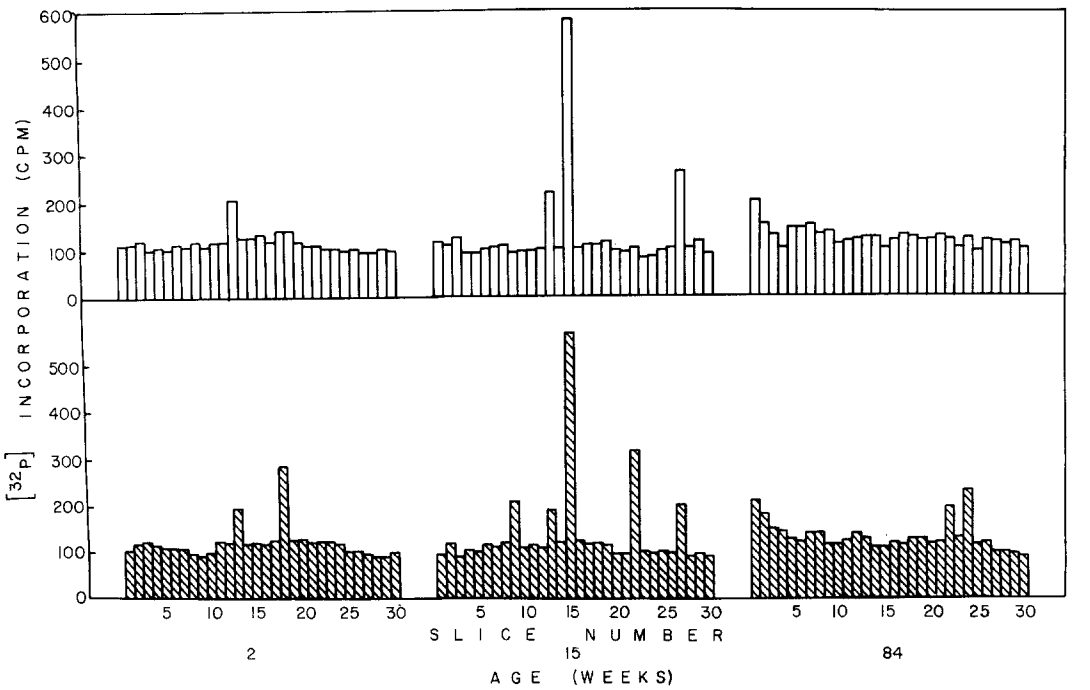

Fig. 2a Effect of calcium on phosphorylation of individual NHC proteins of cerebral cortex of female rats of different ages.

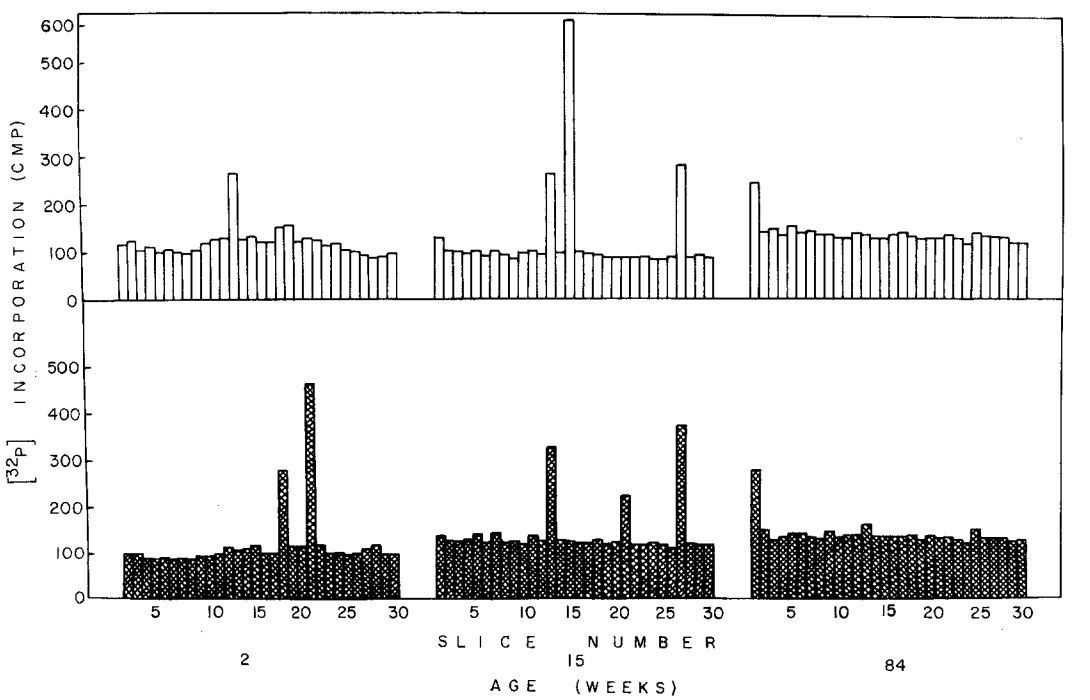

Fig. 2b Effect of 17β-estradiol on phosphorylation of individual NHC proteins of cerebral cortex of female rats of different ages.

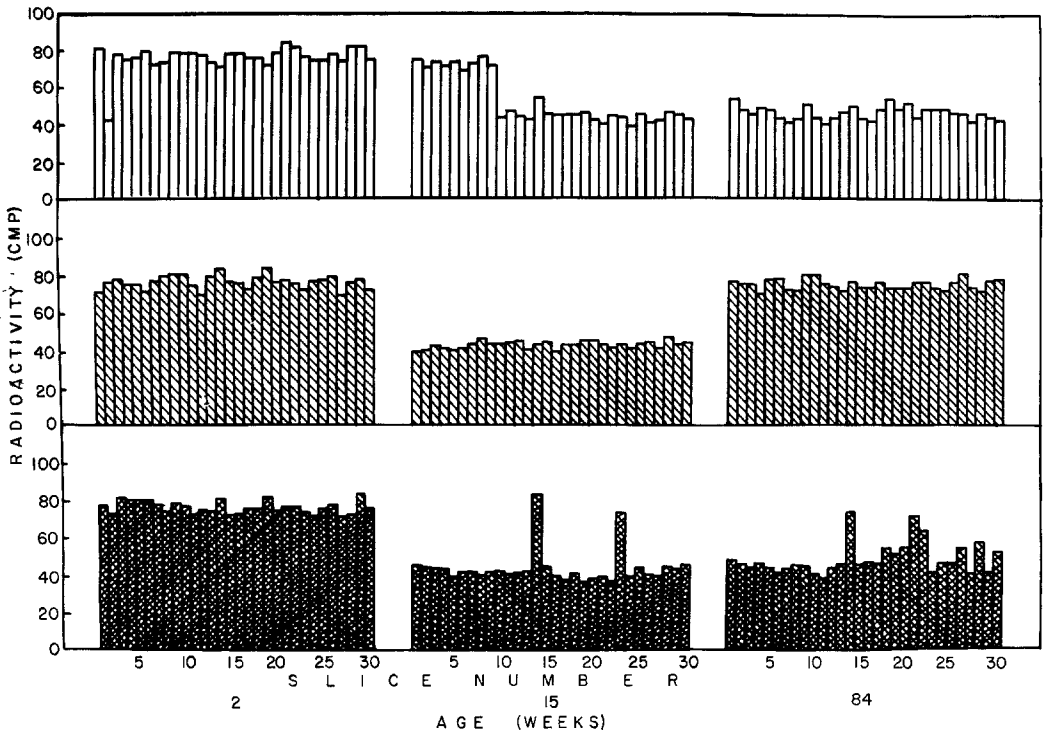

Fig. 3 Effects of calcium and 17β-estradiol on acetylation of individual NHC proteins of cerebral cortex of female rats of different ages.

proteins including chromosomal proteins (14,15). Our studies show that calcium not only stimulates phosphorylation of total NHC proteins, but this seems to be due to stimulation of phosphorylation of a few specific NHC proteins. How such differen tial phosphorylation of NHC proteins is achieved is not known.

Qualitatively, the NHCP of the oviduct of an estradiol-treated rat is different from that of an untreated rat (16). The steroid hormone, ecdysone, causes an increase in NHC protein content at specific loci of the chromosomes of the larva of Sciara (17). Phosphorylated NHC proteins have been implicated in gene expression (5,8). The differential phosphorylation of specific NHC proteins by estradiol seen in our studies suggests that the specific effect of the hormone on transcription may be brought about by stimulation of phosphorylation of specific NHC proteins making available specific genes for transcription.

Even though acetylation of histones, particularly of those of the nucleosome, is reported to stimulate transcription (18) and destabilise the nucleosome (19-21),

little is known about the effect of acetylation of NHC proteins on chromatin structure and function. Unlike phosphorylation, no specific NHC protein is acetylated at any age. Calcium does not have any effect on acetylation of any specific NHC proteins, though estradiol stimulates acetylation of a few NHC proteins. It is suggested that acetylation of NHC proteins may be less important for regulation of gene expression than phosphorylation. These studies show that covalent modifications of NHC proteins, particularly phosphorylation, may have significant role in the expression of genes. Endogenous effectors like calcium and estradiol, which not only modulate covalent modifications of NHC proteins but also of histones (22,23), and whose levels are known to alter during the life span, may affect gene expression.

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