Vol. 90, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 12, 1979 Pages 708-714

EFFECTS OF POLYAMINES ON IN VITRO PHOSPHORYLATION AND ACETYLATION OF HISTONES OF THE CEREBRAL CORTEX OF RATS OF VARIOUS AGES

RATNA DAS and M.S. KANUNGO*

Biochemistry Laboratory, Department of Zoology,
Banaras Hindu University, Varanasi 221005, India

Received July 23,1979

SUMMARY - In vitro phosphorylation and acetylation of histones and their modulation by spermine and spermidine were studied using slices of cerebral cortex of female rats of various ages. Phosphorylation and acetylation of individual histones decrease with increasing age. Spermine and spermidine have stimulatory effects on both the modifications of specific histones in immature rats. These effects decrease with increasing age. Such changes in covalent modifications of histones may alter gene expression and contribute to the aging process.

INTRODUCTION - Chromatin is a complex of DNA, histones and non-histone chromosomal (NHC) proteins. Histones being basic in nature, act as non-specific gene repressors and participate in the basic structure of chromatin (1,2). They undergo post-translational covalent modifications such as phosphorylation, acetylation, methylation and ADP-ribosylation, which play significant roles in the modulation of their interaction with the DNA.

Phosphorylation of histones weakens their interaction with the negatively charged DNA, and thereby causes their dissociation from the latter. This may stimulate template activity of DNA for RNA synthesis (3). Acetylation of histones is reported to change the conformation of the chromatin and stimulate its template activity (4). Recently, we have reported that acetylation of histones and RNA synthesis decrease in the brain as a function of age (5). Polyamines are cations. They directly act at the chromatin level by binding with DNA phosphates (6). Hence they

*To whom reprint requests should be addressed.

0006-291X/79/190708-07$01.00/0

may alter the structure of chromatin, and thereby alter its template activity. Polyamines have been implicated in cell multiplication and growth (7). Since neuronal division stops at a very early age in mammals, we wanted to know if polyamines have any effect on phosphorylation and acetylation of histones of the brain of rats which may have a role in cell division.

MATERIALS AND METHODS - Female rats, 2-(immature), 15-(adult) and 84-weeks (old), of Wistar strain were used. They were killed by cervical dislocation and the cerebral cortex was immediately cut into slices of approximately 0.4 mm thickness. In vitro phosphorylation (8) and acetylation (9) of histones were studied by incubating 1.0 g of the sliced tissue in flasks containing 4.0 ml Krebs-Ringer bicarbonate buffer, pH 7.4. Spermine (10^{-5}M) or spermidine (6 x 10^{-5}M) was added to the experimental flasks. Cycloheximide (2 x 10^{-4}M) was added to each flask to inhibit protein synthesis. 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 (carrier free) and 0.1 mCi of (U-^{14}C) sodium acetate (specific activity 49.3 mCi/mmole; Bhabha Atomic Research Centre, Bombay) were added to the flasks set up for phosphorylation and acetylation, respectively. The shaking was continued for 60 min.

The slices were then taken out and washed thrice in cold Krebs-Ringer bicarbonate buffer. The chromatin was purified from the tissue (10) and the histones were extracted fron the chromatin (11). The electrophoretic analysis of histone was done on 10% polyacrylamide gels (7.5 x 0.6 cm) containing 6.25 M urea and 0.9 M acetic acid (12). The histone bands in the gel were scanned at 500 nm. The bands were cut by a blade and the radioactivity in

each slice was counted in an LS-100C Beckman scintillation counter after digesting the slices in 0.2 ml of 30% H_2O_2 (v/v) at 37°C overnight.

RESULTS AND DISCUSSION - The electrophoretic mobilities of all the five histones of the brain of immature, adult and old rats were found to be similar (Fig. 1). This corroborates the findings of Klimenko et al (13), and Carter and Chae (14). Such a similarity in the pattern indicates that apparently no change occurs in the polypeptide chains of histones of the cerebral cortex of rats during the life span.

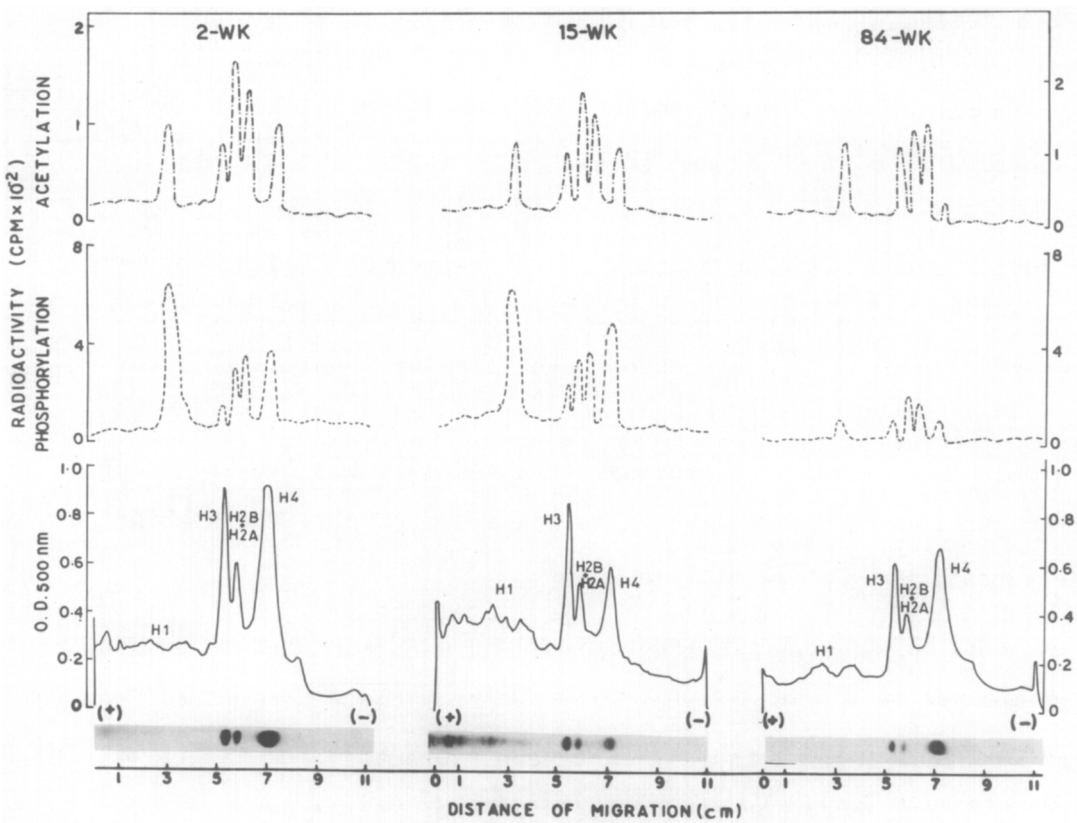

Fig. 1. Densitometric scanning of the histones, and phosphorylation and acetylation of individual histones of the cerebral cortex of female rats of different ages. (———), gel scanning at 500 nm; (— — —), phosphorylation; and (—·—·—) acetylation.

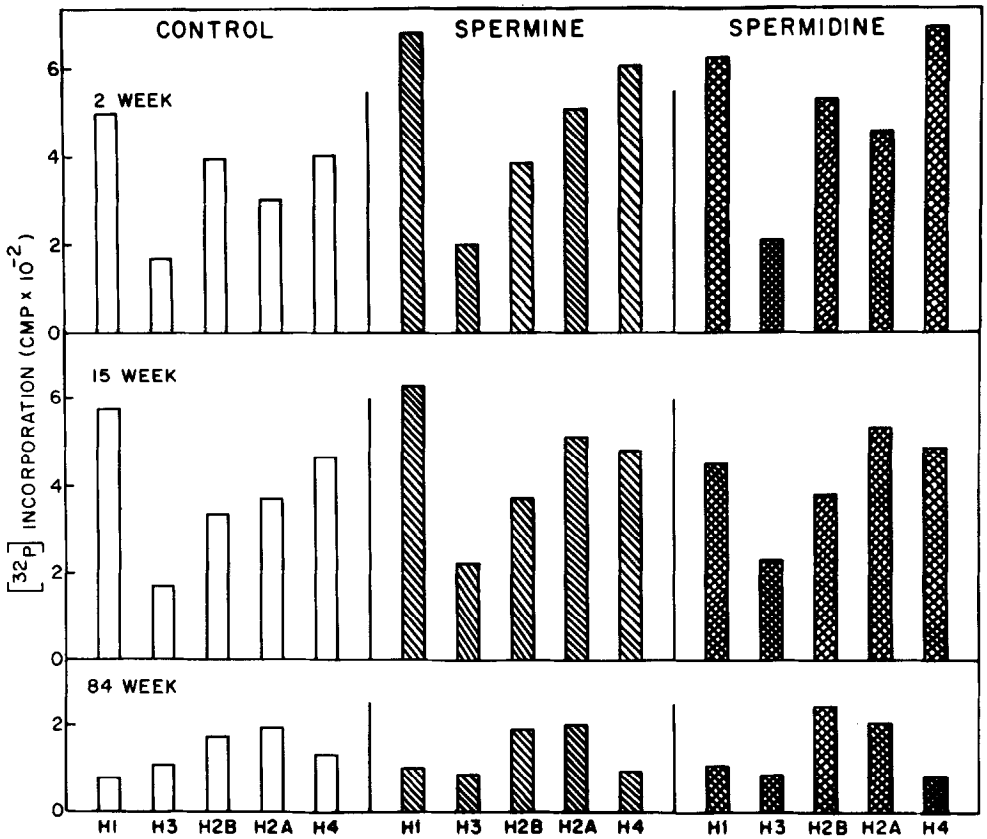

Fig. 2. Effects of polyamines on phosphorylation of individual histones of the cerebral cortex of female rats of three ages.

Normal in vitro phosphorylation of individual histones of the three ages are shown in Figs. 1 and 2. Individual histones are differently phosphorylated as a function of age. The relative degree of ^{32}P incorporation into individual histones of young and adult rats is H1 > H4 > H2A > H2B > H3. Phosphorylation of all the histones decreases in old age, the maximum decrease being in H1 and H4. H1 is present in the linker region and H4 in the nucleosome core. Decrease in phosphorylation of both the histones may be due to conformational changes in the chromatin. Such changes may account for a decrease in the template activity of chromatin as a function of age (5). Polyamines have specific stimulatory effects on

phosphorylation of individual histones in vitro (Fig. 2). Spermine stimulates phosphorylation of H1, H2A and H4 histones in the young, and the effect decreases with increasing age. Spermidine stimulates phosphorylation of H1, H2B, H2A and H4 histones in the young. In the adult, stimulation of phosphorylation is seen only in H2A. No effect on any histone is seen in the old. Differential stimulation of phosphorylation of specific histones by polyamines and its variation with age indicates that these effectors may have specific regulatory effects at the genetic level.

Acetylation of lysine residues of chromosomal proteins is reported to alter the structure and function of chromatin and stimulate DNA-dependent RNA synthesis (4). Our data show that in vitro acetylation of individual histones varies with age (Fig. 1,3). The relative degree of acetylation of individual histones in young and adult rats is H2B > H2A > H1, H3, H4. A high degree of H4 acetylation in immature rats observed by us may be responsible for the high template activity of genes at this age (15). A dramatic decrease in H4 acetylation in old age may cause condensation of chromatin and a decrease in RNA synthesis (16). The decrease in the degree of acetylation of H2B and H4 with increasing age, and the absence of any significant change for H1, H3 and H2A histones is likely to cause changes in the conformation of chromatin at selective sites and also decrease RNA synthesis.

Fig. 3 shows that polyamines stimulate acetylation of the nucleosomal histones, H3, H2B, H2A and H4 in vitro. This effect decreases with age. The effect of H1 histone of immature rats is negligible. Acetylation of H2B and H4 has been shown to be necessary for RNA synthesis (17). Since polyamines are reported to stimulate gene transcription (18), their specific stimulatory

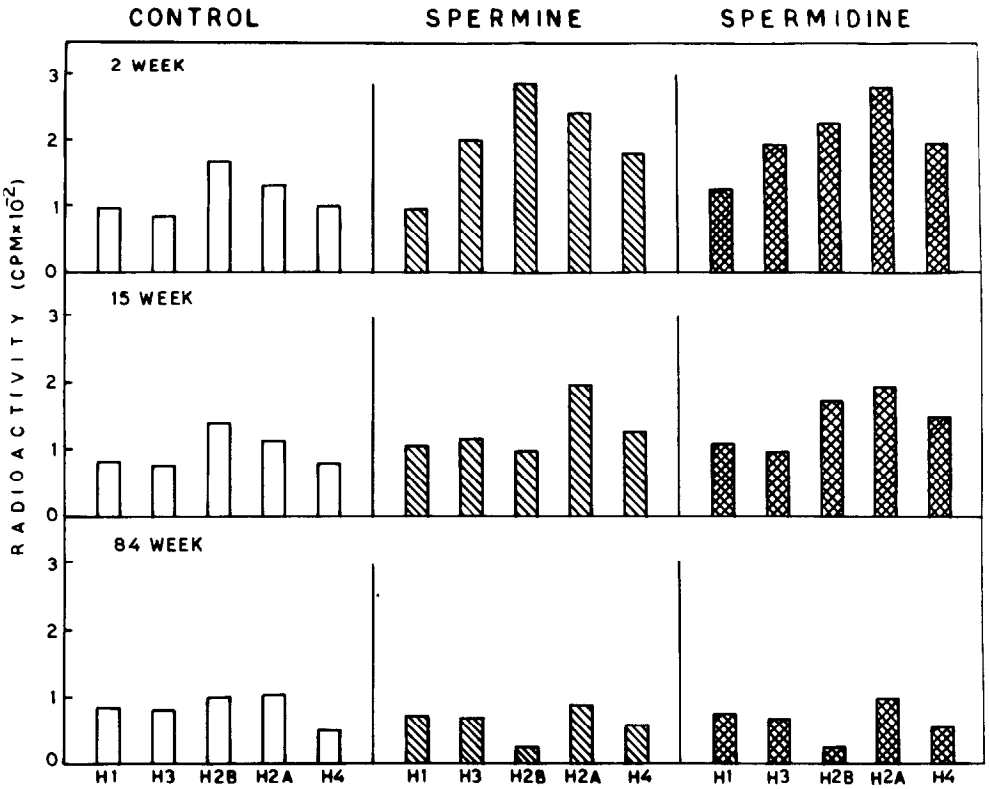

Fig. 3. Effects of polyamines on acetylation of individual histones of the cerebral cortex of three ages.

effects on acetylation of nucleosomal histones, and their alterations with age indicate the specific role that polyamines may have on age-dependent gene expression. Epinephrine and estradiol have also been shown to stimulate acetylation of specific histones *in vitro* (19).

This is the first report which shows that polyamines stimulate phosphorylation and acetylation of specific histones *in vitro*. Such effects may cause modulation of the expression of specific genes. We have observed th at the levels of both spermidine and spermine, but not of putrescine, decrease with age (20). This may cause age-dependent variations in the expression of genes. Such alterations at the level of genes brought about by endogenous effectors in a sequential order after the

attainment of reproductive maturity may lead to senescence. This is consistent with the gene regulation theory proposed by Kanungo (21).

ACKNOWLEDGEMENTS - This investigation was supported by grants from the Nuffield Foundation, England; PL-480 (IN-ARS-24); University Grants Commission and Department of Science & Technology, India, to M.S.K. R.D. Thanks the C.S.I.R. for a research fellowship.

REFERENCES

1. Johns, E.W. & Butler, J.A.V. (1964) Nature 204, 853-855.
2. Butler, J.A.V. (1966) In Histones (Eds. deReuck, A.V.S. & Knight, J.) J. & A. Churchill, London, 4-17.
3. Hnilica, L.S. (1972) In The Structure and Biological Function of Histones, C.R.C. Press, Cleveland, Ohio.
4. Huang, R.C. & Bonner, J. (1962) Proc. Natl. Acad. Sci. U.S.A. 48, 1216-1222.
5. Kanungo, M.S. & Thakur, M.K. (1979) Biochem. Biophys. Res. Commun. 87, 266-271.
6. Tabor, H. (1962) Biochemistry 1, 496-501.
7. Janne, J., Poso, H. & Raina, A. (1978) Biochim. Biophys. Acta 473, 241-293.
8. Kadohama, N. & Anderson, K.M. (1977) Can. J. Biochem. 55, 513-520.
9. Sung, M.T., Harford, J., Bundman, M. & Vidalakas, G. (1977) Biochemistry 16, 279-285.
10. Bonner, J. et al (1968) In Methods in Enzymology (Eds. Grossman, L. & Moldave, K.) Academic Press, New York 12(B), 3-65.
11. Elgin, S.C.R. & Bonner, J. (1970) Biochemistry 9, 4440-4447.
12. Panyim, S. & Chalkley, R. (1969) Arch. Biochem. Biophys. 130, 337-346.
13. Klimenko, A.J., Malishev, A.B., Nikitin, V.N., Paskevich, N.F. & Simirenko, L.L. (1974) Dokl. Akad. Nauk. SSSR, Nov. Ser. 215, 1491-1492.
14. Carter, D.B. & Chae, C.B. (1975) J. Geront. 30, 28-32.
15. Davie, J.R. & Candido, P.M. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 3574-3577.
16. D'Anna, J.A., Tobey, R.A., Barnam, S.S. & Gurley, L.R. (1977) Biochem. Biophys. Res. Commun. 77, 187-194.
17. Allfrey, V.G. (1971) In Histones and Nucleohistones (Ed. Phillips, D.M.P.) Plenum Press, New York, 241-294.
18. Caldarera, C.M., Casti, A., Guarnieri, C. & Moruzzi, G. (1975) Biochem. J. 152, 91-98.
19. Thakur, M.K., Das, R. & Kanungo, M.S. (1978) Biochem. Biophys. Res. Commun. 81, 828-831.
20. Das, R. (1979) "Enzymic changes and modifications of chromosomal proteins in rats as a function of age", Ph.D. Thesis, Banaras Hindu University, Varanasi.
21. Kanungo, M.S. (1975) J. Theor. Biol. 53, 253-261.