Vol. 82, No. 1, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 15, 1978 Pages 223-227

REVERSIBLE EFFECTS OF NA-BUTYRATE ON HISTONE ACETYLATION

by

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Received March 28, 1978

SUMMARY: Histones were labeled by incubating HeLa cells in the presence of radioactive leucine for 20 hours. Following a 5 hour chase in non-radioactive medium the cells were exposed to 7 mM Na-butyrate to increase the level of histone acetylation. Histones were then extracted, fractionated by high-resolution electrophoresis in acetic acid-urea gels and the specific activity of the parental form of H4 histone and that of each acetylated form was calculated. No differences were found in the specific activities indicating that the major effect of butyrate on histone acetyl levels involves histones which were synthesized before the administration of butyrate. The effect is reversible and within 15 minutes after the removal of the drug most of the acetylated forms of H4 histone are converted to the unmodified form.

It has been suggested that the reversible modification of histones by acetylation is part of a complex set of mechanisms which control chromatin structure and influence DNA-template function. Much of the literature on the subject has been the subject of a recent review (1).

We have recently provided evidence that histone acetylation does in fact alter the interaction of these proteins with DNA thus making the DNA more susceptible to digestion with pancreatic DNAse 1 (2). The experiments were conducted with HeLa cells which were exposed to Na-butyrate to augment the level of histone acetylation (3 , 4) and during these studies we discovered that butyrate interacts directly with histone deacetylase (5) causing an inhibition of this enzyme without inhibiting histone acetyl-transferase activities.

The present studies examine the reversibility of the butyrate effect *in vivo* and also test whether butyrate modifies the acetylation of histones which were synthesized before the addition of the drug.

0006-291X/78/0821-0223$01.00/0

MATERIALS AND METHODS.

HeLa S-3 cells were grown at a concentration of 4-6 x 10^5 cells per ml in Minimum Essential Medium (Joklik-modified) (Grand Island Biological Company, Grand Island, N.Y.). In labeling experiments 3 mCi of [4,5,^{3}H]-L-leucine (specific activity 40 Ci/mmole, New England Nuclear,Boston , Mass.) were added to one liter of cells and the incubation continued for 20 hours. The cells were then harvested, washed once with non-radioactive medium and reincubated in the non-radioactive medium for 5 hours at a concentration of 4-6 x 10^5 cells per ml to allow incorporation of the radioactive histones into the chromatin. Butyrate was then added to the cell suspension to obtain a final concentration of 7 mM and part of the incubated cells were harvested after 21 hours and frozen in MEM:glycerol 1:1 (v:v). The remaining cells were washed with butyrate-free medium and incubated in MEM for 15 minutes. After this time the cells were harvested and also frozen in MEM:glycerol 1:1 (v:v). Prior to isolation of nuclei, cells were thawed and washed with cold MEM three times and then resuspended in 10 mM NaCl ; 10 mM Tris-HCl, pH 7.0 ; 3 mM $MgCl_2$ (buffer A) containing 0.5% Nonidet NP-40. After 15 minutes cells were homogenized with a Dounce homogenizer (20 strokes) and the nuclei recovered by centrifugation at 3000 x g for 5 minutes. Each nuclear pellet was then washed three times by homogenization in buffer A and centrifugation. The final pellets were extracted once with 0.4 N H_2SO_4 and histones were precipitated from the extracts with 10 volumes of acetone, washed with acetone, with ether and dried.

Histones were separated by electrophoresis on polyacrylamide gels under conditions where parental and acetylated forms of various histone fractions are resolved as separate bands (6). The gels were stained with 0.1% Amido Black in 35% methanol - 15% acetic acid, and the densitometric tracings of the stained bands were recorded. The bands corresponding to individual subfractions of H4 histone were cut out, and the radioactivity in each band was determined by combusting the gel slices in a Packard Sample Oxidizer and measuring the ^3H_2O activity of the combustion products. The specific activity of each band was determined by dividing the total counts of each band by the µg of histone present in each band.

RESULTS AND DISCUSSION.

In HeLa cells the level of histone acetylation is greatly increased following treatment of the cells with Na-butyrate (3 , 4). Ingram and coworkers have also reported that the incorporation of radioactive precursors into DNA is not immediately stopped after the butyrate is added to the cell suspension but proceeds for several hours at a decreasing rate (4). Since it is well documented that histone synthesis is concomitant with DNA synthesis (7) the question arises as to whether the increased levels of histone acetylation seen in butyrate-treated cells might be due to an accumulation of newly-synthesized histones in their multi-acetylated forms. We planned the following experiments to find out

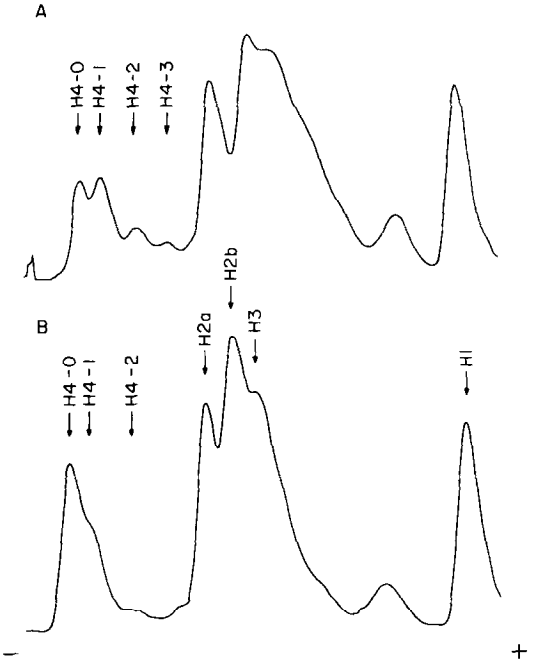

Fig.1. Densitometric tracing of histone distribution in stained gels. Histones were obtained from cells which had been labeled with radioactive leucine and then exposed to Na-butyrate for 21 hours, and from cells labeled with radioactive leucine, exposed to Na-butyrate for 21 hours and then incubated in butyrate-free medium for 15 minutes. Panel A : electrophoretic analysis of histones extracted from butyrate-treated cells ; panel B : electrophoretic analysis of histones extracted from butyrate-withdrawn cells.

whether butyrate would alter the level of acetylation of histones which were made long before the administration of the drug.

Cells were incubated with radioactive leucine to label the histones and then in non-radioactive medium to make sure that all the radioactivity found in histones after the addition of butyrate was confined to the "old histones". After the chase cells were exposed to butyrate (7 mM) for 21 hours. Histones were extracted from the purified nuclei and fractionated by high-resolution electrophoresis on acetic acid-urea-polyacrylamide gels (Fig. 1) (6). The specific activity of H4 histone was calculated for the parental form and for the various acetylated forms. Table I shows that no substantial differences are found among the specific activities of different H4 sub-

TABLE I

Butyrate-induced conversion of non-acetylated histones into acetylated forms

Histone source	Butyrate-treated HeLa cells				Butyrate-withdrawn HeLa cells		
H4 histone subfraction	H4-0	H4-1	H4-2	H4-3	H4-0	H4-1	H4-2
Specific activity (cpm/μg)	281	288	325	337	281	310	296

fractions, indicating that the radioactively-labeled "old" H4 histones had been converted to their acetylated forms after the addition of butyrate.

The effect of butyrate on histone acetylation is also reversible and most of the acetylated forms of histones are transformed back to the parental non-acetylated form within 15 minutes after butyrate is removed from the incubation medium*(Fig. 1). The specific activity of H4 has been also calculated after butyrate removal (Table I) and, within experimental error, no differences can be found when compared to the corresponding specific activities of H4 histone subfractions extracted from cells which were grown in the presence of butyrate. This proves that histones which were acetylated have been converted to non-acetylated forms.

ACKNOWLEDGMENTS.

This research was supported by grants from The National Foundation / March of Dimes (1-440), NIH (GM 17383 and CA 14909), ACS (NP 228 H) and from the Rockefeller Foundation Program for Reproductive Biology.

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* Reversibility of the butyrate effect within 30 minutes has also been observed by G.M. Riggs and V.M. Ingram (personal communication).

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