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ASSOCIATED EFFECTS OF SODIUM BUTYRATE ON HISTONE ACETYLATION AND ESTROGEN RECEPTOR IN THE HUMAN BREAST CANCER CELL LINE MCF-7

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Sodium butyrate at a concentration of 5mM causes significant hyperacetylation of the core histones in the human breast cancer cell line MCF-7. Histone hyperacetylation was achieved in rapidly-growing cells at 40% confluency after 24 hours in 5mM sodium butyrate. More nearly confluent cells did not reach as high a level of histone hyperacetylation. Upon assaying the estrogen receptors, both cytosolic and KCl-extractable nuclear, we found that butyrate treatment had lowered the estrogen receptor levels in both compartments. To our knowledge this is the first report of an effect of sodium butyrate on estrogen receptor levels.

Since the original studies of Allfrey and his co-workers (1-4) describing histone acetylation as a post-translational, reversible event, intensive study of its function has progressed. This has led to the suggestion that histone acetylation is involved in modifying histone-DNA interactions to modulate transcription (5,6). Several laboratories have reported that high levels of histone acetylation make the associated DNA more accessible to DNAase digestion (7,8). The repeated observation that transcriptionally active regions of the DNA are also preferentially digested in limited DNAase digestion (9-13) indicates at least that transcriptionally active and highly acetylated regions of the chromatin are similar in this regard. A key finding was that of Riggs et al. (14) who found that sodium butyrate causes extensive hyperacetylation of histones. This initial finding allowed others to then show that this effect occurs due to inhibition of histone deacetylase enzymes (15,16). We and others have found that histone hyperacetylation had no effect on the overall rate of in vitro transcription (in isolated nuclei) (17,18,19) although others have expressed differing views (20).

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More recently, Reeves and Cserjesi, using sodium butyrate and a different assay method, have found that different species of RNA and protein are produced upon histone hyperacetylation in Friend erythroleukemia cells (21), although others have found conflicting results in HTC (hepatoma tissue culture) cells (22). Oliva and Mezquita (23) and Grimes and Henderson (24) have recently reported that hyperacetylation of histone H4 seems to be correlated most closely, not with transcriptional activity, but with the transition from nucleohistone to nucleoprotamine which occurs upon maturation in spermatogenesis. Reports of Reczek et al. (25) and Perry and Chalkley (26) underscore the idea that the effects of histone hyperacetylation may be due more to higher order (internucleosomal) chromatin structure changes rather than simply to modification of interactions between DNA and histone (intranucleosomal). Perry and Chalkley's work in addition suggests that the apparent greater susceptibility of hyperacetylated chromatin to DNAase digestion may be due to its greater solubility after digestion rather than to greater susceptibility to digestion, throwing open to question whether DNAase digestion susceptibility and transcriptional activity are closely related.

If histone hyperacetylation and transcription are related, one should be able to discern changes in certain protein levels following butyrate treatment, and indeed this has been observed, to include both enhanced and inhibited induction of proteins (27-30). Morita et al. (31) have shown that butyrate causes a reversible elevation of alkaline phosphatase, dependent on RNA and protein synthesis, in the human rectal cancer cell line HRT-18. Littlefield et al. (32) have shown a similar phenomenon in HeLa S3 cells, also reporting that butyrate lowers glucocorticoid receptor levels in HeLa cells and Horwitz et al. have described a similar effect on progesterone receptors in the human breast cancer cell line T-47D (33).

We now report that butyrate treatment lowers the levels of both cytosol and nuclear estrogen receptors, unoccupied and total, in human breast cancer cells in long term tissue culture. To our knowledge, this is the first report of this phenomenon.

MATERIALS AND METHODS

Cell Culture: MCF-7 cells were originally generously supplied by the Breast Cancer Task Force Cell Culture Bank at EG & G Mason Research Institute. They were grown in Corning plastic flasks (150cm^2) in 5% CO_2 in air at 37°C. Growth medium was basal medium Eagle: modified-autoclavable (powdered) plus nonessential amino acids, 2mM L-glutamine, 10% calf serum, 100 units/ml penicillin, 100µg/ml streptomycin (Grand Island Biological Co.), and 0.006µg/ml insulin (Sigma). Cells were harvested by replacing the growth medium with Hank's balanced salt solution without calcium and magnesium, but with 1mM EDTA, incubating for 10 minutes at 37°, and centrifuging. Cells were then washed with the above solution without EDTA at 4° and once with PG buffer (5mM sodium phosphate, 1mM β-mercaptoethanol, 10% glycerol, pH 7.4) at 4°, centrifuged and the pellets frozen at -70°C until use.

Histone Isolation and Electrophoresis: Histones were isolated as previously described (34) and electrophoresed according to the method of Panyim and Chalkley (35) in 25cm 15% polyacrylamide tube gels with 2M urea. Staining was accomplished by overnight incubation at room temperature in 1% (W/V) amido black, 7% (V/V) acetic acid. Gels were then destained by diffusion in 7% acetic acid and densitometric scans performed at 600nm using a Gilford 2520 gel scanner in a Gilford 250 spectrophotometer.

Estrogen Receptor Assays: Estrogen receptor assays were done using the single saturating dose protamine sulfate assay (36) at 4° (non-exchange conditions) and 30° (exchange conditions) to measure unoccupied and total receptors, respectively. The sucrose density gradient assay was utilized also, as described earlier (37).

Isotopes and Chemicals: [^{3}H]-estradiol 17-β was from New England Nuclear or Amersham, electrophoretic reagents from Bio-Rad, except N,N,N',N'-tetramethylethylenediamine (Sigma), and sodium butyrate from Fisher, while Sigma was the source of protamine sulfate. All chemicals were of reagent grade or higher quality.

RESULTS AND DISCUSSION

In order to achieve a level of histone acetylation significantly above that found in control cells we treated MCF-7 cells with sodium butyrate under various conditions. Finding that 40% confluent cells responded better to butyrate than those more nearly confluent, we determined that 5mM butyrate administered to these cells for 24 hours yielded significant histone hyperacetylation, as shown in Figure 1, but had no effect on cell viability. When 9 x 150cm^2 flasks reached an average of 40 percent confluency, the medium was replaced with fresh medium 5mM in sodium butyrate. 1M butyrate, stock solution pH 7.0, was prepared by making 1M butyric acid in H_2O and adjusting to pH 7.0 with NaOH. At zero time, this stock solution was added to growth medium to make it 5mM in butyrate. Control cells (9 x 150cm^2 flasks) merely underwent a change to fresh medium at zero time. At 24 hours cells were harvested and frozen at -70°C. Histones were isolated and analyzed as described earlier in Materials and Methods. Treatment with butyrate

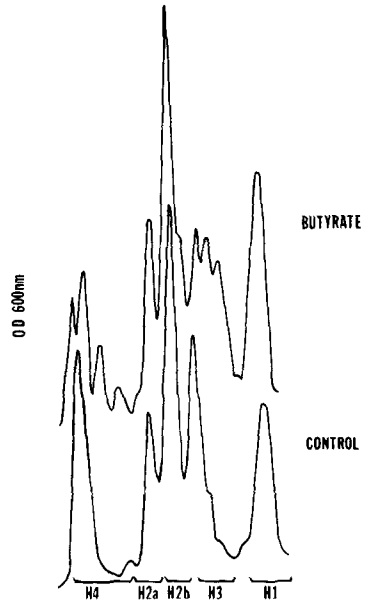

Figure 1 Hyperacetylation of histones with 5mM sodium butyrate for 24 hours. MCF-7 cells in logarithmic growth (40% confluency) were changed from regular medium to the same with 5mM sodium butyrate for 24 hours. Control cells underwent a medium change without sodium butyrate. Histones were then isolated and electrophoresed, stained and destained, as described previously (35) and scanned at 600nm with a Gilford gel scanner. Scans do not coincide exactly because they are from different electrophoretic runs.

resulted in increased percentages of acetylated species of especially histones H4 and H3, which have been found to be the most fundamental histones in nucleosome structure (38,39).

Since hyperacetylation of histones has been reported to cause changes in chromatin structure and function, we felt that perhaps this level of hyperacetylation might somehow affect the level of estrogen receptors in the cytosol and/or KCl-extractable nuclear fractions. To test this, the single saturating dose protamine sulfate assay was performed on cytosol and nuclear extracts, using both exchange and non-exchange conditions. Figure 2 shows the results for unoccupied cytosol and nuclear receptors. Those for total receptors were similar. The results indicate that butyrate treatment and histone hyperacetylation are associated with reduced receptor levels in both cytoplasmic and nuclear compartments, though in some experiments nuclear levels were reduced more than cytoplasmic levels. Figure 3 shows the results

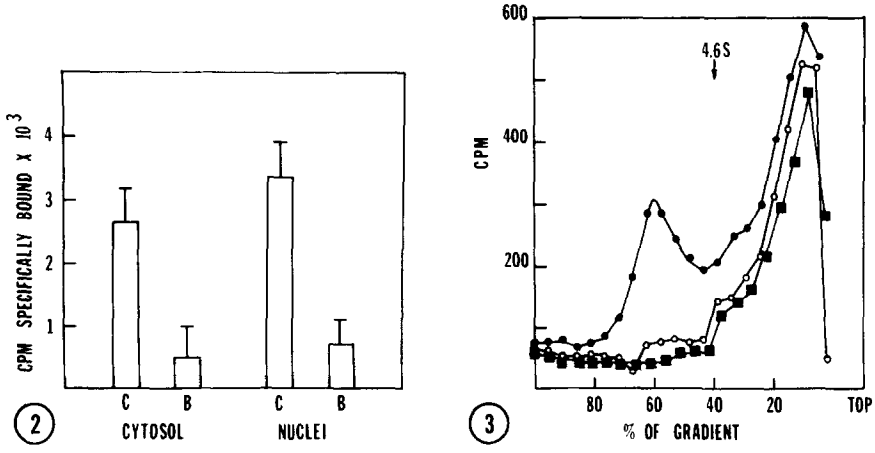

Figure 2 Effect of sodium butyrate (5mM for 24 hours) on estrogen receptor levels. After treatment with butyrate as described in the text, cytosol and KCl-extractable nuclear receptors were assayed with the single saturating dose protamine sulfate assay. Error bars represent standard deviations for three samples from the pooled cytosol or nuclear extract from three 150 cm^2 (surface area) flasks of cells. Representative of 10 experiments.

Figure 3 Sucrose density gradient analysis of the effect of butyrate on cytosol estrogen receptor levels. ● , control low salt (no KCl) gradient analysis of 230µl of cytosol labelled with 10^{-8}M [^{3}H]-estradiol 17β for 4 hours at 4°C before removing non-bound label with dextran-coated charcoal. ○ , cytosol from butyrate-treated cells. ■ , non-specific binding, determined by incubation of samples identical to the above with 10^{-6}M diethylstilbestrol and 10^{-8}M [^{3}H]-estradiol 17β. These cytosol samples are from a different experiment than those in figure 2. Representative of 3 experiments.

of a separate experiment with another passage of cells in which cytosol receptor levels were compared using the sucrose density gradient assay. Here again, butyrate treatment resulted in lowering of the estrogen receptor levels, a similar phenomenon occurring with KCl-extractable crude nuclear receptors assayed with sucrose gradients.

There are many possible explanations for this phenomenon, four of which will be considered. The first is that butyrate has somehow enhanced the production of a phosphatase, as in HeLa cells (32) and HRT-18 cells (31) which dephosphorylates the estrogen receptor, rendering it unable to bind estrogen. This possible effect of butyrate may or may not be related to histone hyperacetylation. Another possibility is that the histone hyperacetylation has opened up new sites for the estrogen receptor in the residual nuclear fraction, which sites are too tight for loosening with 0.6M KCl. We feel this is un-

likely, however, since this was found not to be the case for the glucocorticoid receptor in HeLa cells (32). A third intriguing possibility is that butyrate treatment might be causing acetylation of the estrogen receptor itself, rendering it unable to bind estrogen. Finally, since butyrate is known to be a reasonably efficient synchronizer of cells into the G1 phase (40) the lowering of estrogen receptors may simply be a cell-cycle dependent phenomenon. All four of these possibilities are under investigation in our laboratory.

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