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n-Butyrate and dexamethasone synergistically modulate the surface expression of epidermal growth factor receptors in cultured rat hepatocytes

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n-Butyrate was previously found to increase the epidermal growth factor (EGF) receptor binding in primary cultures of rat hepatocytes. We show here that butyrate and dexamethasone synergistically modulate the surface expression of the EGF receptors. The butyrate-induced enhancement of high-affinity EGF binding was only slight in the absence of glucocorticoid, but was strongly and dose-dependently amplified by dexamethasone. Butyrate counteracted the inhibition by insulin of the dexamethasone-induced increase in EGF binding. The results indicate that the glucocorticoid has a permissive effect on a butyrate-sensitive process that determines the surface expression of the high-affinity class of EGF receptors.

EGF receptor; Butyrate, *n*-; Dexamethasone; (Cultured rat hepatocyte)

1. INTRODUCTION

Epidermal growth factor (EGF) is a strong mitogen [1] that acts through specific transmembrane receptors [2]. In many cells the surface expression of EGF receptors has been found to be related to the state of cell differentiation [3–5]. The number of EGF receptors can be increased by agents capable of inducing morphological differentiation in cultured cells, such as cAMP [6] and retinoids [3,7], and also by glucocorticoids [8]. *n*-Butyrate has profound effects on many cultured cells. This agent may preserve an apparently differentiated morphology, inhibit growth and cause selective alterations in gene expression [9]. We have recently reported that butyrate up-regulates EGF receptors in primary cultures of rat hepatocytes [10]. In particular, butyrate preserved the high-affinity receptor subpopulation [10] which is present on freshly isolated hepatocytes [11]. Since glucocorticoids have also been shown to increase EGF binding in hepatocytes [12], and dexamethasone was routinely present in the medium in [10], it was of interest to examine the relationship between the glucocorticoid-induced and the butyrate-induced effects on EGF receptors. We report here that the two agents act synergistically, dexamethasone exerting a strong permissive effect on the ability of butyrate to enhance the surface expression of EGF receptors in cultured hepatocytes.

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2. MATERIALS AND METHODS

2.1. *Materials*

^{125}I-EGF (spec. act. 105–132 Ci/g) was from Amersham International (Buckinghamshire, England). Unlabeled EGF (receptor grade) was from Collaborative Research, Inc. (Bedford, MA). Sodium *n*-butyrate, dexamethasone, bovine serum albumin (fraction V) and collagen (rat tail, type VII) were from Sigma (St. Louis, MO). Insulin was from Novo (Copenhagen, Denmark).

2.2. *Hepatocyte isolation and culture*

Male Wistar rats (180–220 g) were fed ad libitum. Hepatocytes were isolated as described [11] and seeded into

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Costar plastic culture wells (12-well dishes) with collagen in a 1 : 100 dilution [13] at cell densities of 25 000 cells/cm^2. The cells were cultured without serum in a 1 : 1 combination of Dulbecco's modified Eagle's medium with low glucose (5.6 mM) and Waymouth's medium MAB 87/3 (Gibco, Grand Island, NY) modified as described [14], supplemented with penicillin (67 μg/ml) and streptomycin (100 μg/ml). The medium was not changed during the culture period. *n*-Butyrate and dexamethasone at the indicated concentrations were added 2–3 h after plating. Insulin (100 nM) was added at the time of plating.

2.3. *^{125}I-EGF binding assays*

The monolayers were washed twice with ice-cold Krebs-Hepes-Ringer buffer with 1% bovine albumin, pH 7.4 (binding buffer). For estimation of surface binding the cells were chilled on ice for 30 min, and incubated with ^{125}I-EGF in binding buffer to a total volume of 0.6 ml per well at 0°C for 20 h. For saturation binding analysis labeled EGF was diluted with unlabeled EGF in a fixed ratio of 1 : 9 as described [11]. After removing the medium and rinsing the monolayers four times the cells were dissolved in 0.5 N NaOH for 2 h at room temperature. The radioactivity in the medium (free ligand) and in the dissolved cells (bound ligand) was measured separately in a gamma counter. The nonspecific binding was estimated by measuring the binding in the presence of 300 nM unlabeled EGF. Protein was determined according to Lowry et al. [15]. EGF binding activity was expressed as fmol EGF/mg cell protein. Data for Scatchard analysis were analyzed by the LIGAND computer program [16], and the best fit of the data to a one-site or a two-site model was evaluated by the *F* statistics test incorporated in the program.

3. RESULTS

The decline of EGF binding in hepatocytes occurring during culture [12] can be restored by butyrate [10]. Fig.1 shows the effect of butyrate on the high-affinity EGF binding in the absence or presence of 250 nM dexamethasone. The EGF binding was determined at a low ligand concentration (20 pM labeled EGF) to permit binding predominantly to the high-affinity receptor class [10]. In the absence of the glucocorticoid, butyrate had only a slight and inconstant effect on the EGF binding. In the presence of dexamethasone, however, butyrate dose-dependently (1–5 mM) increased the EGF binding (fig.1A). In addition, the dose-dependent increase of EGF binding caused by dexamethasone [12] was also markedly potentiated by 5 mM butyrate (fig.2A). Insulin (100 nM) inhibited the dexamethasone-induced increase in EGF binding (fig.2) as described [12]. Insulin also slightly depressed the EGF receptor binding in butyrate-treated cells (figs 1,2). It should be noted, however, that butyrate to a large extent abolished

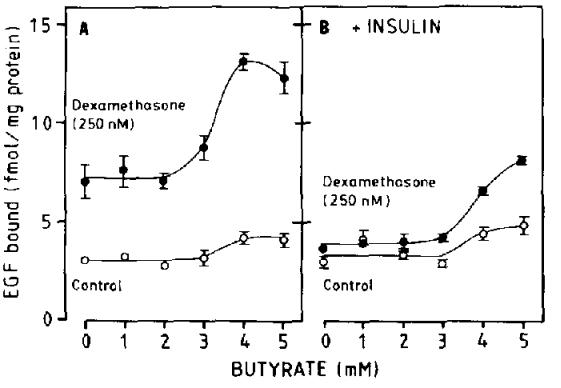

Fig.1. Dose-dependent effects of butyrate on the high-affinity EGF receptor binding in the absence and presence of dexamethasone. Rat hepatocytes were cultured in medium without insulin (A) or with 100 nM insulin (B). Dexamethasone (250 nM) and varying concentrations of butyrate were added 2 h after plating when indicated. After 42 h in culture the cells were incubated at 0°C for 20 h with 20 pM labeled EGF. Each point represents the mean ± SE of three cultures.

the inhibition by insulin [12] of the dexamethasone-induced receptor up-regulation (fig.2B).

Scatchard analysis of the EGF binding to cells cultured in the presence of insulin (100 nM) (fig.3A) or insulin (100 nM) plus dexamethasone (250 nM) (fig.3B) revealed linear binding plots,

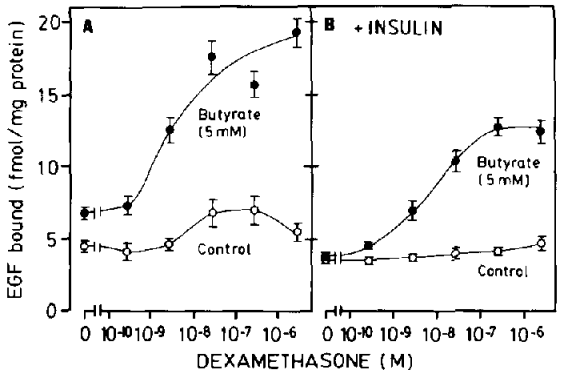

Fig.2. Dose-dependent effects of dexamethasone on EGF receptor binding in the absence and presence of butyrate. Hepatocytes were cultured in medium without insulin (A) or with 100 nM insulin (B). Butyrate (5 mM) and varying concentrations of dexamethasone were added 3 h after plating as indicated. EGF binding was determined after 42 h in culture, using 20 pM labeled EGF. Each point represents the mean ± SE of six cultures from two experiments.

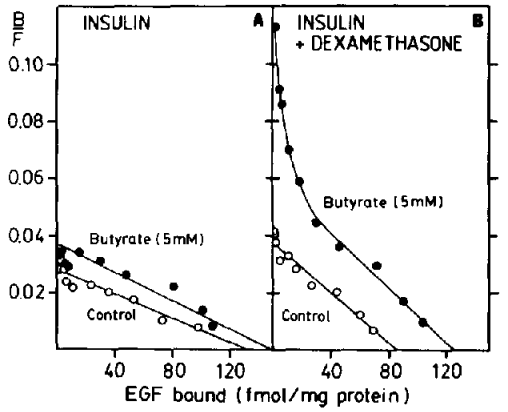

Fig.3. Scatchard analysis of EGF binding. Hepatocytes were cultured in medium supplemented with 100 nM insulin (A) or 100 nM insulin + 250 nM dexamethasone (B). Butyrate (5 mM) was added 3 h after plating of the cells as indicated. After 42 h in culture, the cells were assayed for EGF binding as described in section 2. Each point represents the mean of two cultures. One of three experiments.

suggesting the presence of a single binding activity in these cells. When 5 mM butyrate was added 2–3 h after plating, curvilinear binding plots suggesting the presence of different affinity states of the receptor emerged in hepatocytes treated with dexamethasone and insulin, but not if dexamethasone was excluded. Computer (LIGAND) analysis of data from three independent experiments on cells exposed to butyrate in the presence of dexamethasone and insulin showed that a two-site model was a better fit than a one-site model (P = 0.008), with a high-affinity receptor population with K_d = 0.011 nM comprising about 4% of the total receptors, and a low-affinity population with K_d = 0.37 nM. In cells cultured with insulin in the absence of dexamethasone, butyrate did not affect the affinity of EGF for its receptor, the K_d being 0.99 nM and 0.98 nM in the absence and presence of butyrate, respectively. Addition of dexamethasone to insulin-treated cells in the absence of butyrate very slightly increased the affinity (from K_d = 0.99 nM to K_d = 0.54 nM).

4. DISCUSSION

As a growth factor receptor tyrosine kinase showing homology with the *erb*B-1 oncogene product, the EGF receptor has been widely appreciated to play an important role in cell proliferation [1,2]. Changes in the EGF receptor status, which can occur through transcriptional control [17], receptor internalization/externalization [18], or changes in affinity [19], may confer upon the cell new patterns of growth responsiveness. In liver, several reports have focused on the regulation of the EGF receptor. The receptor number declines both in regenerating liver after partial hepatectomy [20] and in hepatocytes plated in primary culture [12]. Lin et al., who studied hormonal modulation of EGF binding to hepatocytes in primary culture, showed that glucocorticoids increase the EGF binding and that this increase is inhibited by insulin [12]. We have recently found that *n*-butyrate enhances the EGF binding in cultured hepatocytes [10]. The present report demonstrates that there is a marked synergism between the glucocorticoid dexamethasone and butyrate in the up-regulation of surface EGF receptors, particularly the high-affinity binding, in cultured hepatocytes. Dexamethasone was required for the effect of butyrate, and butyrate increased the effect of dexamethasone and counteracted the inhibition by insulin on the dexamethasone-induced up-regulation of the EGF receptors.

The molecular mechanisms responsible for the observed interaction between dexamethasone and butyrate are not known. Both dexamethasone and butyrate have been found to inhibit hepatocyte DNA synthesis [10,21] and promote differentiated characteristics in primary hepatocyte culture [21,22]. Butyrate affects a large number of nuclear processes and causes histone hyperacetylation, but the precise mechanisms responsible for the effects of butyrate have not been resolved. Certain observations may suggest an interaction between the effects of butyrate and those of glucocorticoids at the level of gene transcription. Both butyrate and dexamethasone induce the metallothionein-I gene in hepatoma cells [23] and increase the number of β-adrenoceptors in the 3T3-L1 preadipocyte system [24]. Butyrate also increases the capacity of dexamethasone to induce the alkaline phosphatase activity of HeLa S_3 cells [25] but inhibits the expression of certain glucocorticoid-inducible gene products in HTC cells [26]. Taken together, these reports may suggest converging effects of glucocorticoids and butyrate at the level of gene transcription. The present results indicate that in

hepatocytes butyrate and dexamethasone synergistically affect the EGF receptor status, particularly increasing the high-affinity receptor subpopulation. Although it is conceivable that butyrate affects transcription of the EGF receptor gene, it is also possible that it acts through a transcription-dependent process that indirectly modulates the EGF receptor properties, such as the aggregation state [19] or phosphorylation [2,27], which may alter receptor affinity.

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