Opposite Effects of Sodium Butyrate on CCK mRNA and CCK Peptide Levels in RIN Cells

Colette Roche,1 Martine Cordier-Bussat,1 Christelle Ratineau,1 Christine Bernard,1 Jacques Philippe,2 and Jean-Claude Cuber1

1INSERM U45, Pavillon Hbis, Hôpital E. HERRIOT, 69437 Lyon Cedex 03, France; and 2Centre Médical Universitaire, Laboratoire de Microbiologie, Geneva, Switzerland

The effects of the differentiation-inducing agent sodium butyrate on cholecystokinin (CCK) expression was investigated in the pancreatic islet tumor cell line RIN 1056E, which contains high levels of CCK-like immunoreactivity (CCK-LI). Exposure to butyrate for 24 h dose-dependently inhibited cell proliferation and increased the cell content in CCK-LI over the concentration range 0.1–8 mM. With 2 mM butyrate, cell proliferation was decreased by 50% and CCK-LI content was increased by 300%, whereas the level of steady-state CCK mRNA was reduced by 75%. Cycloheximide (10 μg/mL) abolished the sodium butyrate-induced increase in CCK-LI content. This article reports the novel finding that butyrate exerts opposite effects on CCK mRNA and immunoreactivity. The butyrate-induced increase in cellular CCK-LI content is entirely dependent on continuing protein synthesis.

Key Words: Butyrate; CCK immunoreactivity; CCK mRNA; (RIN 1056E cells).

Introduction

Peptide hormone production is an example of differentiated function that may require the coordinate regulation of a number of correlated genes, such as the one encoding the peptide of interest and those involved in the proteolytic processing, packaging, and secretion. Sodium butyrate is a differentiation-inducing agent that stimulates insulin gene transcription and increases insulin cell content and secretion in cultured insulin-producing cell lines, such as the transplantable rat insulinoma (RIN) (Philippe et al., 1987a; Karlsen et al., 1991). Similarly, butyrate stimulates somatostatin gene expression and secretion in RIN cells (Green and Shields, 1984). In contrast, butyrate was shown to inhibit gene transcription in other systems (Philippe et al., 1987a; Lazar, 1990; Ormandy et al., 1992). This was accompanied by a reduction in the level of the corresponding posttranslational products (Lazar, 1990; Ormandy et al., 1992). Taken together, these data indicate that butyrate-induced modifications of gene expression are accompanied by a parallel variation in the level of posttranslational products. The present article reports that butyrate may exert anticoordinate effects on gene expression and accumulation of posttranslational products. The mechanism was evidenced in a cell line that produces cholecystokinin (CCK), a regulatory peptide of the brain–gut axis displaying a variety of effects on several gut and brain functions. Butyrate decreased the steady-state CCK mRNA levels and increased the cellular content in CCK-like immunoreactivity (CCK-LI). This last effect was shown to be entirely dependent on continuing protein synthesis.

Results

Cells incubated for 24 h with increasing concentrations of sodium butyrate over the range 0.1–8 mM showed a decreased proliferation rate, as assessed by cell DNA content (Fig. 1A). This decrease in cell DNA content became significant with 0.5 mM sodium butyrate. A more than 50% decrease was obtained with 8 mM sodium butyrate. At the same time, a clear dose-dependent enhancement of cellular CCK-LI was observed (Fig. 1B). For subsequent experiments, we used 2 mM of sodium butyrate, which produced the half-maximal effects on cell proliferation and CCK-LI content. The effects of 2 mM sodium butyrate were recorded during 24 h following addition of the drug. The increase of cellular CCK-LI became significant only 24 h after sodium butyrate treatment (Fig. 2A), whereas CCK secretion in the culture medium was never modified on sodium butyrate treatment (Fig. 2B).
To investigate whether the effects of sodium butyrate on cellular CCK-LI were related to an increase in CCK gene expression, cells were incubated with 2 mM sodium butyrate for various time lengths. Total RNA was subsequently extracted and analyzed by Northern blot. As shown in Fig. 3, the Northern blot analysis revealed a single band of CCK mRNA, which was approx 750 nucleotides in size. With 2 mM butyrate, no significant changes in CCK mRNA levels were observed until 6 h of incubation. In cells exposed for 12 h and 24 h to sodium butyrate, the CCK mRNA levels were reduced by 60 and 75%, respectively, with little additional effect between 24 and 48 h (Fig. 3).

To investigate the mechanism involved in the sodium butyrate-induced increase in CCK cell content, cells were incubated with either 2 mM butyrate alone or a mixture of

---

Fig. 1. Effects of increasing concentrations of sodium butyrate on DNA synthesis (A), cellular content of CCK-LI (B), and CCK release (C) in rat islet tumor RIN 1056E cells. The RIN cells were exposed to sodium butyrate for 24 h and then treated for cell proliferation and CCK-LI measurements as described in Materials and Methods. All results are corrected for amount of DNA/culture well and expressed as mean values ± SEM (n = 6). *p < 0.02 vs control, **p < 0.01 vs control.

Fig. 2. Time-course study of sodium butyrate effects on cellular CCK-LI content (A) and CCK release (B). Cells were exposed to sodium butyrate (2 mM) for 6, 12, and 24 h. Control cells: open squares; sodium butyrate-treated cells: full circles. All results are corrected for amount of DNA/culture well and expressed as mean values ± SEM (n = 6). *p < 0.01 vs control, NS (not significant).

Fig. 3. Time-course study of sodium butyrate effects on CCK mRNA levels. Cells were exposed to sodium butyrate treatment for 6, 12, 24, and 48 h. Top: Representative Northern blot (A = β-actin). Bottom: Densitometric quantification of CCK mRNA levels after normalization to β-actin. Results are mean ± SEM of five independent experiments.