PARADOXICAL EFFECTS OF SODIUM BUTYRATE ON THE GLUCOCORTICOID INDUCTIONS OF GLUTAMINE SYNTHETASE AND GLYCEROL PHOSPHATE DEHYDROGENASE IN C6 CELLS

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1. Introduction

Glycerol phosphate dehydrogenase (GPDH) and glutamine synthetase are both localized in glial cells of the central nervous system [1,2]. Moreover, both enzymes are induced by hydrocortisone (HC) in C6 glioma cells, and both inductions require de novo RNA and protein synthesis [3,4]. In a variety of cell types, sodium butyrate (NaB) is a potent inducer of differentiation, enzyme activity and new gene expression [5,7]. Since NaB also interacts with GPDH induction in glial cells [8] as well as with other HC-mediated enzyme inductions in different cell types [9], we are continuing to utilize this short-chain fatty acid to clarify the mechanisms by which glucocorticoids regulate gene expression in brain cells.

We have reported that 5 mM NaB completely inhibits the HC-mediated rise in GPDH activity in both C6 glioma and primary cultures of oligodendrocytes [8]. This inhibition is reversible and is not the result of NaB-induced non-specific toxicity. Furthermore, NaB does not inactivate either basal or induced GPDH molecules. We have also shown that the HC induction of GPDH is mediated by the classical steroid–receptor complex and is solely due to an increase in the rate of synthesis of the enzyme [9,10]. To investigate the nature of the inhibition of this induction by NaB, we measured the rate of synthesis of newly made GPDH molecules and examined the effect of NaB upon another glucocorticoid-inducible enzyme in C6 cells, glutamine synthetase (GS). We now report a novel finding. By itself, NaB induces GS. However, in combination with HC, NaB has no secondary effects upon the HC-mediated induction of GS. This lack of effect contrasts with our earlier report of a complete block of the HC induction of GPDH.

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2. Materials and methods

C6 glioma cells are a clonal cell line originally derived from rat neural tumor induced by *N*-nitromethylurea [11]. Cells were cultured and maintained as in [8].

Glutamine synthetase was determined by a radiochemical assay [4]. Protein was determined on the cytosol fraction [12]. The rate of synthesis of GPDH under various treatment conditions was determined by immunoprecipitation and SDS gel electrophoresis as in [10].

3. Results and discussion

Treatment of C6 glioma cells with 5 mM NaB results in a 3–4-fold increase in GS activity after 24 h (fig.1). In combination with 1.38 μM HC, the response is equal to the sum of the enzyme activities (above basal level) induced by NaB and HC individually (fig.1). Actinomycin D (1 μg/ml) and cycloheximide (30 μM) abolished the NaB-mediated induction, while 0.1 μg/ml actinomycin D was ineffective (not shown). Thus, NaB, like HC, requires RNA and protein synthesis in order to induce GS. That the antiglucocorticoid progesterone (10 μM) inhibits the HC-mediated induction of GS activity (fig.1), but not that of NaB, suggests that these agents induce GS by partially different mechanisms. The dose–response curve of the NaB induction of GS is bell-shaped (fig.2) with a

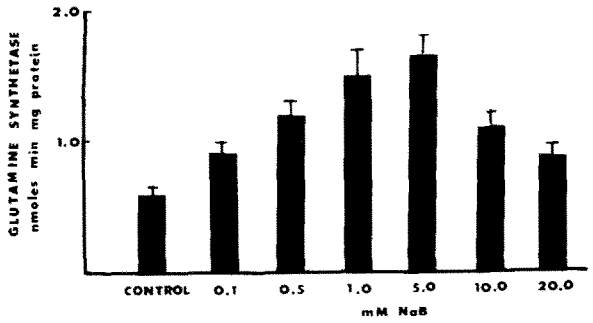

Fig.1. Confluent C6 glioma cells grown on Falcon plastic 25 cm^2 flasks were induced for 24 h under the various treatment conditions as shown. Cells were harvested, and glutamine synthetase activity is determined. HC was 1.38 μM; NaB, 5 mM; progesterone (Prog), 10 μM. The values expressed are av. 3 flasks ± SD. The experiment was repeated 3 times.

maximum at 1–5 mM NaB. Doses as low as 0.1 mM NaB result in small, but significant increases in GS activity.

While it is clear that NaB affects at least one of the steps of the HC induction of GPDH, it appears that NaB has absolutely no effect on the HC induction of glutamine synthetase. We base this conclusion on the following: By itself, 5 mM NaB yields a maximal GS response (fig.2), while 1.38 μM HC, by itself, represents a saturating concentration in terms of optimum GS induction in C6 cells [13]. Hence, if NaB has a secondary effect upon the HC-mediated induction, one would not obtain an additive GS response when C6 cells are exposed to these two agents simultaneously. However, this is not the case. Induced enzyme levels are indeed additive (fig.1).

This differential action of NaB on GS *vs* GPDH, combined with the observation that progesterone fails to inhibit the NaB induction of GS while profoundly inhibiting that of HC, suggests that NaB does not interact with glucocorticoid steroid receptors. We had reported the absence of a NaB effect upon cytoplasmic steroid binding in C6 cells [8]. Moreover, if any other steps by which the steroid–hormone complex exerts its biological effects in C6 cells are impaired by NaB, then one should expect inhibition of HC-inducible proteins other than GPDH. Since NaB does not inhibit glutamine synthetase induction by HC, it appears that the NaB inhibition specific to GPDH induction occurs at those steps of its induction (that it does not share with GS induction) distal to the glucocorticoid–receptor complex reaching the nucleus. Our observation of a total inhibition in the

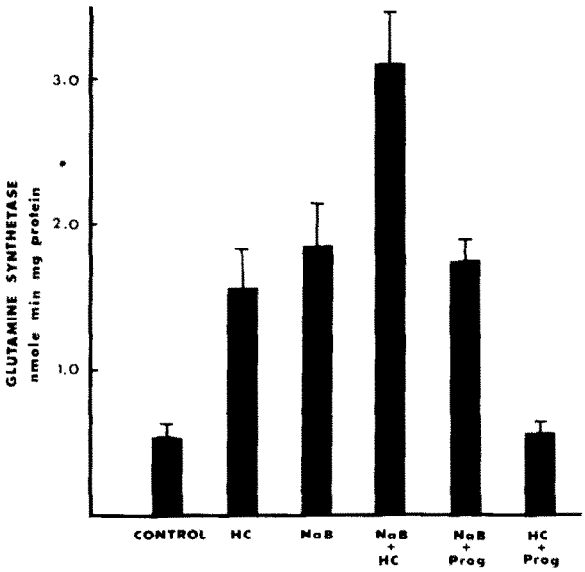

Fig.2. Confluent C6 glioma cells grown on Falcon plastic 25 cm^2 flasks were treated with various [NaB] for 24 h. Cells were then harvested and glutamine synthetase activity determined. Glutamine synthetase activity is expressed as nmol glutamine . min^{-1} . mg $protein^{-1}$. The values expressed are av. 3 flasks ± SD. The experiment was repeated 2 times.

induced increase in GPDH synthesis by NaB (table 1) also supports the notion of a nuclear site of inhibition. The extent of this inhibition correlates well with the complete inhibition of induced enzyme activity. Further investigation of the step(s) of GPDH induction uniquely inhibited by NaB should provide us with a more specific understanding of the molecular mechanisms of hormone action.

In searching for a mechanism at the nuclear level to explain NaB's varied actions on these two enzymes, the well-known massive histone hyperacetylation by NaB in mammalian cells [14,16] is currently a fruitful area of research. It has been postulated that histone acetylation correlates with gene activation [17]. The observed NaB induction of GS is in accord with this theory. Nevertheless, it remains to be seen whether the amount of enzyme induction correlates with the extent of histone modification. NaB at 0.5 mM (fig.2) yields nearly maximal GS induction, while recent studies of histone hyperacetylation [14,16] employed higher doses (1–10 mM). In this regard the less than maximal GS levels with >5 mM NaB is perplexing, though non-specific toxicity at these higher concentrations is a real possibility. Among the gene products thus far investigated,

Table 1
The relative rates of synthesis of GPDH in response to HC and NaB and their combined action

	Units/GPDH	cpm in GPDH	Total cpm	%Relative rate of GPDH synthesis
	mg protein	mg protein	mg protein	
Control	117	474	1 809 892	0.026
NaB	105	261	1 744 767	0.015
HC	324	4967	2 182 797	0.228
NaB + HC	120	335	1 696 970	0.020

The relative rate of synthesis of GPDH in C6 glioma cells expressed as cpm specifically incorporated into GPDH relative to the total cpm incorporated into protein. Cells were pulsed for 1 h with 5 μCi/ml [^{3}H]leucine 23 h after the addition of the various treatment conditions, where HC was 1.38 μM and NaB was 5 mM. One unit of GPDH activity is expressed as that amount which oxidizes 1 nmol NaOH/min at 30°C

increases in their relative rates of syntheses by NaB seem to be the general rule [6,14,16]. Thus our original observation of inhibition by NaB of the HC induction of GPDH appears exceptional. Since its publication, a strikingly similar inhibition by NaB of estradiol's induction of ovalbumin and transferrin in oviduct has been reported [18]. In this case the site of inhibition is transcriptional, the estradiol–receptor system is unaffected, and the extent of inhibition correlates with the extent of histone acetylation by various other inhibitory short-chain fatty acids.

However, the differential sensitivities of GPDH and GS to sodium butyrate as demonstrated by their dose–response curves may be indicative of separate mechanisms by which NaB acts to inhibit HC-mediated GPDH induction and to induce GS. Whereas 0.1 mM NaB is ineffective in blocking GPDH induction [8], this concentration is sufficient for GS induction (fig.2). Thus, at present a single mechanism such as histone hyperacetylation does not appear to account for NaB's diversity of effects in C6 cells. With respect to glial cells as target tissues for glucocorticoids, only future research will indicate whether these differential actions of NaB on glucocorticoid-mediated enzyme inductions are unique to glial cells or represent a more widespread cellular phenomenon.

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