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THE EFFECT OF GLUCOCORTICOID HORMONES ON RELEASE OF HBsAg FROM PLC/PRF/5 (ALEXANDER) HEPATOMA CELLS

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Summary. — The glucocorticoid hormones dexamethasone, betamethasone, and cortisone enhanced HBsAg release from PLC/PRF/5 cells by 40 per cent, 40 per cent and 10 per cent respectively, as compared to untreated controls. In the same experiments dexamethasone and betamethasone depressed the viable cell population by 20 per cent compared to the controls whereas cortisone had no significant effect. Immune electron microscope studies of the HBsAg material revealed no morphological differences compared to the control. Thus the glucocorticoid hormones dexamethasone, betamethasone and cortisone induce quantitative enhancement of HBsAg release without significant qualitative change. It is suggested that quantitative change in HBsAg release relates at least in part to the cell cycle of PLC/PRF/5 cells.

Key words: *HBsAg release*; *PLC/PRF/5 cells*; *glucocorticoids*; *cell cycle*

Introduction

PLC/PRF/5 is a hepatoma cell line derived from a Mozambican male whose serum contained hepatitis B surface antigen (HBsAg) (Alexander *et al.*, 1976; Macnab *et al.*, 1976; Alexander *et al.*, 1978). The cells release HBsAg in the form of "22nm" particles and filaments, but studies of the supernatant fluid and the cells have failed to provide evidence for the morphologically intact virion (Dane particle) or hepatitis B infectivity (Stannard and Alexander, 1977; Alexander *et al.*, 1978; Daemer *et al.*, 1980; Tabor *et al.*, 1981). Nevertheless, the biochemical and antigenic properties of PLC/PRF/5 show a marked similarity to *in vivo* hepatocellular carcinoma (Gerber *et al.*, 1981) and the cells therefore provide a valuable *in vitro* system for the study of viral infection and malignancy in general and hepatocellular carcinoma in particular.

Attempts to elucidate the control of HBsAg synthesis in PLC/PRF/5 cells using a number of drugs (Daemer *et al.*, 1980; Oefinger *et al.*, 1981) have failed to induce the production of Dane particles or their related antigens, and to date only one compound, the glucocorticoid dexamethasone, has enhanced HBsAg release (Oefinger *et al.*, 1981). The aim of this study was

to extend the findings of Oefinger *et al.*, (1981) by determining (1) whether other glucocorticoid hormones could stimulate HBsAg release by PLC/PRF/5 cells; (2) whether enhancement of HBsAg release by glucocorticoid hormones altered the morphology or ratio of the HBsAg positive particles; and (3) whether there was any relationship between drug enhancement of HBsAg release and the dynamics of cell counts.

Materials and Methods

Drugs and cells. The three glucocorticoid steroids, dexamethasone, betamethasone, and cortisone chosen for this study were obtained from the Sigma Chemical Company, St. Louis, U.S.A. Together they span the range of glucocorticoid activity, with dexamethasone having high activity, betamethasone having moderately high activity and cortisone having low activity (Lerner *et al.*, 1964).

PLC/PRF/5 cells were obtained from Professor N. Stanley of the Department of Microbiology, University of Western Australia. The cells were grown in Eagle's minimal essential medium (Gibco) supplemented with 5 per cent (v/v) foetal calf serum, 0.16 per cent sodium bicarbonate (w/v), penicillin (100 units/ml) and streptomycin (100 μg/ml). For a given experiment equal volumes of cell suspension from the same stock bottle were inoculated into four 25 cm^2 Costar 3050 tissue culture flasks to give a final volume of 10 ml per flask. RIA tests for HbsAg (see below) showed that the variation in HBsAg released from bottle to bottle into the medium was minimal.

Experimental procedure. Cells were grown to about 70 per cent confluency, the medium discarded and exactly 9.8 ml of fresh medium added to each bottle. Fresh medium (0.2 ml) containing, respectively, no hormones (control), dexamethasone, betamethasone and cortisone was then added to each of the four flasks so that the final concentration of hormone was $5.1 \times \times 10^{-5}$ mol/l. The hormones were prepared fresh before each experiment by dissolving them in the culture medium with vigorous shaking for at least $1^1/_2$ hr. Preliminary experiments had established that this concentration of dexamethasone enhanced HBsAg release without evidence of cellular toxicity. The cells were maintained at 37 °C for an average of five days, the medium collected, and clarified by centrifugation at 2,000 rev/min (average of about 400 g) for 15 min. Supernatant fluids obtained from this step were tested for the presence of HBsAg by RIA and for particulate material by electron microscopy. Nine replicates of the experiment were carried out using five separate passages of the PLC/PRF/5 cell line.

RIA for HBsAg. Quantitative analysis of relative amounts of HBsAg was carried out by solid-phase radioimmunoassay (Ausria II, Abbott Laboratories, North Chicago, U.S.A.) using overnight incubation at room temperature. Since this system gives a non-linear relationship between HBsAg present and bound radioactivity (Ling and Overby, 1972), a standard curve was prepared for each experiment to convert counts to relative HBsAg concentrations. The curve was based on 1 : 4, 1 : 2, 3 : 4 and undiluted samples of the dexamethasone treated supernatant fluid. The relative concentrations of HBsAg in all the supernatant fluids were then evaluated using 1 : 2 dilution of these fluids; dilution ensured their count rates fitted well onto the curve. A given Ausria kit was used for a given experiment.

IEM for HBsAg. Qualitative analysis of HBsAg particles was carried out by immune electron microscopy (IEM) as follows: 3.5 ml of the clarified medium was concentrated by ultracentrifugation using a Beckman SW60 titanium rotor at 55,000 rev/min (average of about 300,000 g) for four hr at 4 °C on a Beckman L5-65 ultracentrifuge. The supernatant was discarded and the pellet resuspended in 100 μl of sterile PBS to which 20 μl of anti-HBsAg (Behringwerke) was added. The mixture was placed at 34 °C for one hour, overnight at 4 °C, then centrifuged as above at 50,000 rev/min (average of about 250,000 g). The supernatant was discarded and the pellet resuspended in a drop of PBS. After negative staining with 3 per cent phosphotungstic acid (pH 7) on Forwar-carbon coated grids, it was then examined, using a Philips 301 electron microscope. Immune complexes were photographed and a total of 100 to 600 particles, based on at least three separate experiments, examined for each hormone and the control. Preliminary experiments indicated the complexes detected were not present in the anti-HBsAg serum.

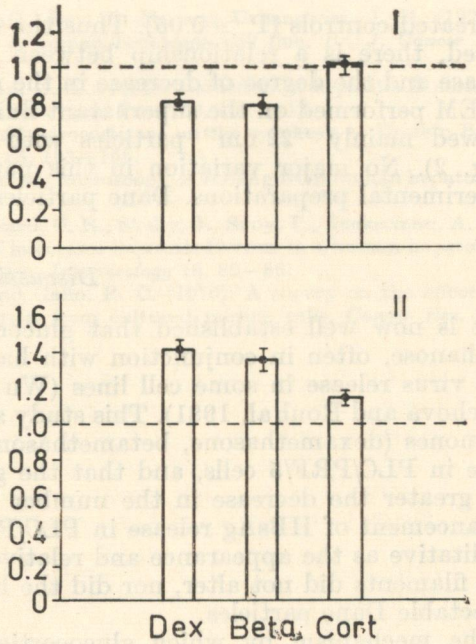

Fig. 1.

Effect of glucocorticoids on HBsAg release (II) and cell number (I)
All values were divided by their respective control value to give controls a value of 1 arbitrary unit. Each column represent the average for the nine experiments with its standard error.
Ordinates: HBsAg release (arbit. units). Interrupted line: control level.

Cell counts. After the supernate had been removed, the cells were washed with Hank's balanced salt solution (Ca, Mg free), trypsinized and resuspended in medium containing trypan blue. Identical volumes of solutions were used in a given experiment. Viable (trypan blue negative) cells were counted, using a Hausser Scientific counting chamber. At least 1000 cells per flask were counted under code.

Results

The effect of the three glucocorticoids on the quantity of HBsAg present in the supernatant fluid is shown in Fig. 1. Under the conditions of the experiment, dexamethasone and betamethasone enhanced detection of HBsAg by an average of approximately 40 per cent each and cortisone by 10 per cent compared to the control. Comparisons between pairs of results using Student's t-test showed that the HBsAg concentration after adding each hormone was statistically significantly greater than the control value ($P < 0.01$). There was no statistically significant difference between the HBsAg values obtained after addition of dexamethasone or betamethasone, ($P > 0.05$) but the HBsAg values for both these hormones were statistically significantly greater than following addition of cortisone ($P < 0.01$).

Fig. 1 also shows the relative effects of the hormones on cell numbers in the nine experiments. Dexamethasone and betamethasone reduced cell number by a statistically significant amount ($P < 0.05$) of about 20 per cent compared to the control. The cell numbers obtained following cortisone treatment were not statistically significantly different from those found in

untreated controls ($P > 0.05$). Thus the results show that for the hormones tested, there is a relationship between the degree of increase in HBsAg release and the degree of decrease in the number of viable cells.

IEM performed on the supernatant fluid from untreated PLC/PRF/5 cells showed mainly "22 nm" particles with occasional filamentous structures (Fig. 2). No major variation in this pattern was observed in any of the experimental preparations. Dane particles were not detected.

Discussion

It is now well established that glucocorticoid hormones, notably dexamethanose, often in conjunction with halogenated pyrimidines, can stimulate virus release in some cell lines (Wu *et al.*, 1976; Bronson *et al.*, 1978; Prachová and Roubal, 1981). This study shows that a range of glucocorticoid hormones (dexamethasone, betamethasone, cortisone) stimulate HBsAg release in PLC/PRF/5 cells, and that the greater the stimulation of HBsAg, the greater the decrease in the number of viable cells. The glucocorticoid enhancement of HBsAg release in PLC/PRF/5 cells is quantitative and not qualitative as the appearance and relative proportions of "22 nm" particles and filaments did not alter, nor did the hormones induce the production of detectable Dane particles.

The mechanism by which glucocorticoids stimulate HBsAg release in PLC/PRF/5 cells is unclear, although a relationship between cell cycle changes and the enhancing effects of dexamethasone and betamethasone, at least, is indicated. The nature of these changes and their significance are currently under investigation.

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