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Increase of virus yields and releases of Borna disease virus from persistently infected cells

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Summary

Borna disease virus grows to low titres in persistently infected cells with an infectious particle to cell ratio of 0.01 to 0.05. Inclusion of *n*-butyrate in the growth medium enhances infectivity yields up to 1 log. This effect is time and concentration dependent. In hypertonic medium with an excess of NaCl, KCl or Na_2SO_4 up to 50% of the total infectious virus yield is released from the cells. Released supernatant virus (buoyant density in sucrose $\rho = 1.22$ g/cm^3) is more heat stabile than cell-bound virus ($\rho = 1.18$ g/cm^3). The access to cell-free (released) virus opens new possibilities for the characterization of this neurotropic agent.

Borna disease virus, *n*-butyrate, virus release

Borna disease (BD) virus persistently infects a variety of tissue culture cells without causing cytopathic changes. Some of its biological and physico-chemical properties indicate that it belongs to the conventional enveloped, RNA-containing viruses. Infectious virus with relatively low virus yields could only be obtained by ultrasonic disruption of infected cells or brains (Hirano et al., 1983; Kao et al., 1984). This suggested a strong association of the agent with cell compartments and hampered attempts to characterize the virus (Ludwig et al., 1973; Ludwig and Becht, 1977; Danner et al., 1978; Herzog and Rott, 1980; Hirano et al., 1983).

To increase virus titres in persistently infected cells we included in the growth medium a variety of chemicals which are known to influence the production of infectious virus or specific proteins (Oldstone and Fujinami, 1982; Bégin, 1980; Leder and Leder, 1975; Saemundson et al., 1980; Hudewentz et al., 1980; Nuss et al., 1975; England et al., 1975).

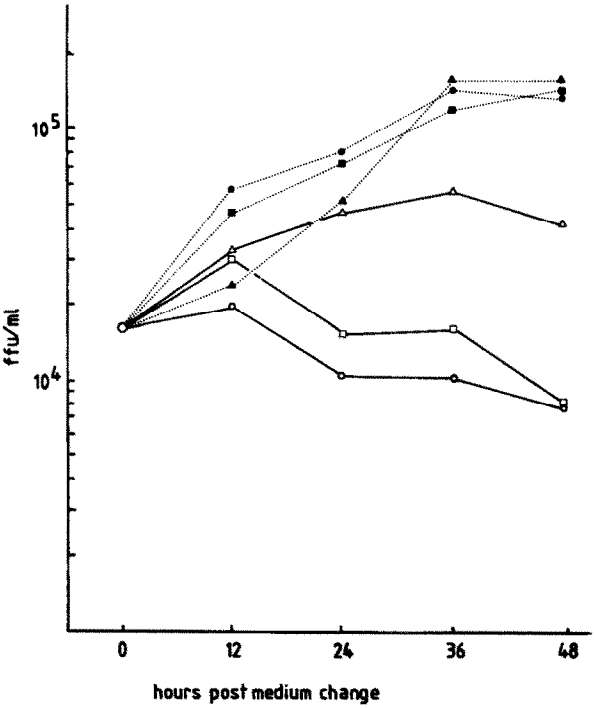

Fig. 1. Effect of sodium *n*-butyrate on production of BD virus. 5×10^5 persistently infected cells of the RKGP (a rabbit kidney cell) cell line were seeded on 5 cm petri dishes in Eagles Medium Dulbecco's modification (EDM) containing 5% inactivated newborn calf serum. After incubation (24 h; 37°C) medium was renewed with *n*-butyrate containing EDM (○, control without *n*-butyrate; □, 2 mM; △ 4 mM; ●, 6 mM; ■, 8 mM; ▲, 10 mM *n*-butyrate) and the cells were further incubated at 37°C. At the times indicated the cells were disrupted by ultrasonication and the clarified supernatant tested for infectivity as described recently (Hirano et al., 1983).

n-Butyrate (6–10 mM) gave rise to a ten-fold increase of BD virus infectivity approximately 36 h after medium change. This effect was time and concentration dependent (Fig. 1). In a variety of cells persistently infected with BD virus which originated from several animal species and man (Table 1) a comparable enhancement of infectious virus was found. The fact that *n*-butyrate is known to inhibit cell division (Wright, 1973), which was also seen in our experiments (Table 1), indicates that the elevated titres are due to an increase of infectious virus particles per cell after *n*-butyrate treatment.

Although the mechanism of action of this drug is not understood, one can speculate that cellular functions controlling the viral genome are altered in such a way that viral RNA- and/or protein synthesis is increased. Such phenomena have been reported for Epstein-Barr virus-infected cells (Kallin et al., 1979; Luka et al., 1979; Saemundson et al., 1980; Hudewentz et al., 1980) and for several enzymes (Kruh, 1982).

Based on reports that under increased salt concentrations viral RNAs were more efficiently translated than cellular ones (England et al., 1975; Nuss et al., 1975), we tested whether elevated salt concentrations in the growth medium would influence BD virus production. Preliminary experiments showed that no significant increase of cell-bound virus was detectable. Surprisingly, however, up to 3 logs more infectious virus could be found in the supernatant than under normal growth conditions. Detailed investigations revealed that the release of virus was salt concentration dependent. High virus titres were found in the supernatant under 90–150 mM excess of NaCl or KCl (Fig. 2). Using Na_2SO_4, maximal virus release was obtained already at concentrations of 60 mM (Fig. 2). These effects were found both in *n*-butyrate-treated and untreated cell cultures.

Time course experiments showed that virus release is a rapid event which seems to be mainly time and less temperature dependent (data not given). After an incubation period of 10 min 30–50% of the virus yield, obtained after prolonged incubation was present in the supernatant. Maximal titres were reached 1–2 h after increase of the salt concentration. From virus yields measured in the supernatant and in the disrupted cells we calculated that up to 50% of the infectious virus could be released.

The viability of the cells is not affected by *n*-butyrate or salt treatment, since after replenishing with medium normal growth was observed. To rule out that virus

TABLE 1

INDUCTION OF BD VIRUS IN PERSISTENTLY INFECTED CELLS

Cell	Species	Medium	FFU/plate	Cells/plate	FFU/cell	Treated/ untreated
CL/TL	cat	EDM	9×10^3	7.2×10^5	0.013	24
		EDMB	6×10^4	2.0×10^5	0.3	
C6/TL	rat	EDM	4×10^2	2.0×10^6	0.0002	54
		EDMB	6×10^3	5.5×10^5	0.011	
MA104/TL	monkey	EDM	2×10^3	8.0×10^5	0.0025	6.3
		EDMB	5.7×10^3	3.6×10^5	0.016	
ML/TL	mink	EDM	7×10^2	8.0×10^5	0.0009	8.3
		EDMB	6×10^3	8.0×10^5	0.0075	
PS/TL	swine	EDM	1.7×10^4	1.4×10^6	0.012	5.7
		EDMB	5×10^4	7.2×10^5	0.069	
Oligo/TL	man	EDM	4.3×10^4	1.0×10^6	0.043	3.3
		EDMB	5.6×10^4	4.0×10^5	0.14	
Cat/TL	cat	EDM	1.4×10^3	1.2×10^6	0.0012	4.0
		EDMB	1.9×10^3	4.0×10^5	0.0048	
RKGP	rabbit	EDM	5.3×10^4	1.0×10^6	0.053	4.3
		EDMB	1.6×10^5	7.0×10^5	0.21	

Approximately 5×10^5 cells of permanent cell lines which are kept in our laboratory and which are persistently infected with BD virus were seeded on 5 cm petri dishes and incubated for 24 h at 37°C, followed by a medium change. Parallel cultures received either EDM or EDM supplemented with 8 mM *n*-butyrate (EDMB). 48 h later cell associated virus was titrated by a focus assay as described recently (Hirano et al., 1983). For calculation of the infectious virus per cell, parallel cultures were trypsinized and the cells counted in a cell counter.

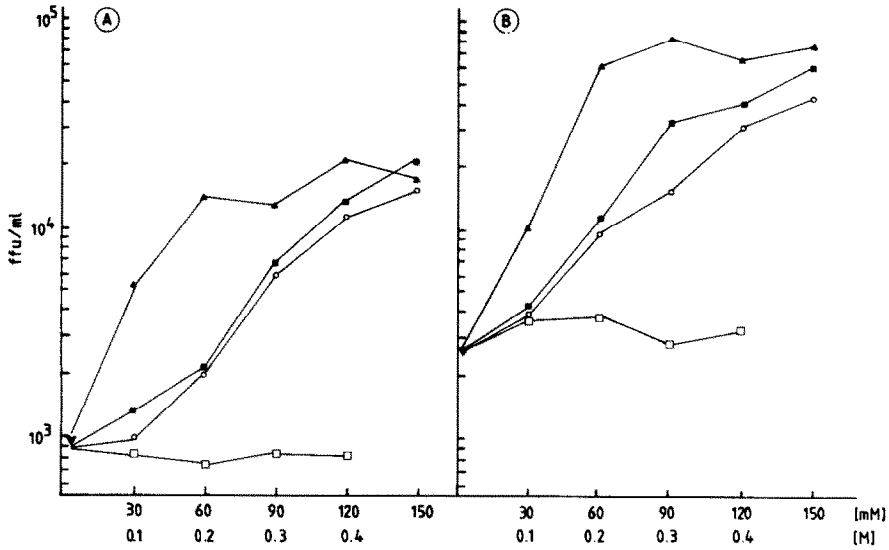

Fig. 2. Release of virus by increasing the salt concentration in the medium. RKGP cells were grown in EDM (A) or in EDMB (B) medium (see legend to table 1) for 48 h. The salt or sucrose concentrations were increased as indicated: □, 0.1–0.4 M sucrose; ○, NaCl; ■, KCl; ▲, Na_2SO_4; 30–150 mM. After 2 h of incubation at 37°C supernatant virus was titrated on rabbit brain cells (Hirano et al., 1983).

release was due to an osmotic shock, elevated sucrose concentrations in the medium (0.1–0.4 M) were employed. No increase of infectious virus could be determined under these conditions (Fig. 2).

In further experiments salt released virus and cell bound virus were compared in some biological and biophysical properties. Although a difference in the buoyant densities in sucrose gradients were evident for both virus preparations (released virus $\rho = 1.22$ g/cm^3, cell bound virus $\rho = 1.18$ g/cm^3; these experiments were repeated several times) no differences in growth behaviour of such viruses in rabbit brain cells were obvious. After an eclipse phase of 24 h newly synthesized virus could be measured followed by the logarithmic growth phase with doubling time of virus between 8 and 10 h and a plateau after 80–120 h post infection.

Inactivation kinetics of released and cell bound virus indicated that the half life of released virus is considerably longer. The higher sensitivity of cell bound virus to inactivation may be due to proteolytic enzymes which are set free by disruption of cells.

The results presented in this report have several implications: First, the possibility to increase virus titres and to release BD virus from cells without ultrasonication offers new approaches for the characterization of the virus. Second, purification of virus followed by radioactive labelling in vitro should allow the identification of viral components by biochemical and electronmicroscopical methods, since experiments to achieve labelling of cell bound virus in vivo and to separate it from cell compartments have reproducibly failed.

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