Sodium valproate, an anticonvulsant drug, stimulates human cytomegalovirus replication

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Valproic acid (VPA), a simple branched-chain fatty acid having anticonvulsant activity and used in the treatment of many forms of epilepsy, markedly stimulated human cytomegalovirus (HCMV) replication in human fibroblasts (MRC-5 cells). The maximum level of stimulation was reached when cells were treated for 24 h before infection. The enhancement of virus replication correlated with an increase in the number of immediate early (IE) and early (E) antigen-positive cells. VPA also induced expression of IE antigens after transfection of fibroblasts with a plasmid containing the entire IE1–2 region. Moreover, VPA stimulated the HCMV IE1–2 promoter/enhancer-mediated expression of β-galactosidase in a stably transfected Jurkat T cell line. Recently, VPA was shown to inhibit glutathione reductase in human red blood cells, but an action through the glutathione metabolic pathway can be eliminated in this case, since VPA decreased the intracellular level of glutathione in Jurkat T cells but not in MRC-5 cells. The ability of VPA to stimulate HCMV replication provides an attractive model for studying the molecular mechanism of the regulation of HCMV IE1–2 gene expression.

Introduction

A primary infection with human cytomegalovirus (HCMV), a human β-herpesvirus, usually results in the establishment of latent infection without overt disease (Alford & Britt, 1990). The latent virus is reactivated under conditions of immunosuppression and sometimes causes devastating disorders such as interstitial pneumonia or retinitis in the transplanted host or in patients infected with human immunodeficiency virus (HIV) (Drew, 1988). The mechanisms involved in the establishment of latency and reactivation of latent HCMV are not yet understood. In a study intended to examine the mechanisms by which HCMV gene expression may be regulated, we looked into the effects of valproic acid (VPA; n-dipropylacetic acid) on HCMV replication and reactivation.

VPA is a simple, branched-chain fatty acid that has broad spectrum anticonvulsant activity and is used in the treatment of many forms of epilepsy (Chapman et al., 1982). However, the use of the drug has occasionally been associated with significant adverse effects (Dreifuss et al., 1987). Small numbers of patients develop serious liver damage and Cotariu et al. (1990) provided evidence that rat liver glutathione reductase (GR) activity is depressed in a dose-dependent manner following administration of VPA. They also showed (Cotariu et al., 1992) that GR, one of the enzymes of the glutathione redox cycle which plays a salient role in maintaining appropriate cellular levels of reduced glutathione, is inhibited in vitro in human red blood cells and in vivo in children’s red blood cells. Glutathione in its reduced (GSH) and oxidized (GSSG) forms, is the major thiol redox system of the cell, providing protection against oxidative damage. In a previous study (Simon et al., 1994), we reported that VPA was able to stimulate HIV replication in acutely and chronically infected cells, and enhanced HIV LTR-directed gene expression. Modification of the GSH intracellular level having been implicated in the regulation of HIV transcription and expression (Buhl et al., 1989; Staal et al., 1990; Roederer et al., 1990; Kalebic et al., 1991), we hypothesized that VPA could act by modifying the intracellular redox balance, which has been shown to regulate activation of various transcription factors such as NF-κB (Toledano & Leonard, 1991; Israël et al., 1992). DNA-binding sequences for NF-κB are present within the HIV LTR (Nabel & Baltimore, 1987) but also within the HCMV promoter (Mach et al., 1989; Sambucetti et al., 1989). Therefore, we asked whether VPA, perhaps by modifying

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the intracellular ratio of GSH/GSSG, was able to activate HCMV replication.

We observed, and report here, that VPA markedly stimulated HCMV replication in human fibroblasts. The enhancement of virus replication correlated with an increase in the expression of immediate early (IE) antigens. In a stably transfected Jurkat T cell line, VPA stimulated the HCMV IE1-2 promoter/enhancer-mediated expression of β-galactosidase. However, it appeared that VPA probably did not act on glutathione metabolism in this case. We discuss various hypotheses about its mechanism of action.

Methods

Cell cultures and reagents. The human lymphoblastoid Jurkat T cell line was grown in RPMI-1640 medium (Gibco). The human fetal lung diploid fibroblast cell line (MRC-5), purchased from Bio-Mérieux, was used to culture the AD169 strain of HCMV. It was maintained as a monolayer culture in MEM (Seromed). All culture media were supplemented with 10% fetal calf serum (FCS; Biological Industries), 2 mM-glutamine (Seromed), 100 IU/ml penicillin and 100 μg/ml streptomycin.

VPA (Sigma) was diluted in culture medium. G-418 sulphate (Gibco) was used at a concentration of 1 mg/ml. In all experiments, cell viability was monitored using the trypan blue dye exclusion test and by the MTT (3-(4,5-dimethylthiazol-2-yl) 2,5 diphenyltetrazolium bromide) test (Mossman, 1983).

Virus titration. Titration of HCMV (AD169 strain) was carried out on MRC-5 cells grown in four-well plates. Cells were treated with various doses of VPA for 24 h. The drug was then removed and the cells were infected with 1 ml of HCMV stock at the desired concentration. Adsorption was performed by centrifugation at 4000 r.p.m. for 45 min at 20 °C. The medium was removed and the cells were fed with 1 ml fresh medium and incubated at 37 °C in a 5% CO₂ atmosphere. Plates were frozen at –80 °C, 3, 5 or 7 days after infection. After thawing and scraping, the presence of infectious HCMV in the cellular lysate was assayed on MRC-5 cells grown in 96-well plates, viral adsorption was performed by centrifugation at 4000 r.p.m. at 20 °C, for 45 min. The supernatant was removed, cells were incubated in complete fresh medium and the appearance of CPE was monitored. The viral yield is expressed in TCID₅₀/ml and was calculated according to the method of Reed & Muench (1938).

Expression of viral antigens. Infection of VPA-treated MRC-5 cells with HCMV was carried out in 96-well plates according to the procedure described above. IE and early (E) antigens were detected, respectively, 24 and 48 h post-infection (p.i.) using an indirect immunoperoxidase assay, on acetone-fixed cells. Mouse monoclonal antibodies (MAbs) E13 and 2A2 to HCMV IE and E antigens, respectively, were purchased from Clonatec (Paris, France). After treatment of fixed cells with 50 μl of MAb E13 or MAb 2A2 (dilution 1:40) for 30 min at 37 °C, cells were washed four times with PBS and then incubated with 50 μl peroxidase-labelled anti-mouse IgG (H + L; dilution 1:40) obtained from Dianova (Düsseldorf, Germany). After 30 min at 37 °C and washing, the HCMV IE or E antigens were detected using 3′,3′-diaminobenzidine chloride (DAB) as substrate for a colorimetric method, in the presence of hydrogen peroxide. Labelled cells were counted with the aid of a light microscope.

MRC-5 transfection procedure. The plasmid pRR47 (5.1 kb) was a gift from Dr Th. Slumming (Erlangen). This plasmid contained the entire IE1-2 gene from the AD169 strain of HCMV (promoter/enhancer plus coding sequences) introduced into pUC18 (EcoRI-SalI). Sub-confluent MRC-5 cells in 96-well microtitre plates were transfected with 400 ng of pRR47 plasmid by the calcium phosphate procedure (Graham & Van der Eb, 1973). Twenty-four hours after transfection, cells were washed twice with PBS. Detection of cells expressing IE antigen was carried out 48 h post-transfection, using the indirect immunoperoxidase assay as described above.

Establishment of the pCMV-LacZ Jurkat T cell line. Jurkat T cells (2×10⁶) were transfected with the pLC-2 plasmid (15, 30 or 50 μg) by an electroporation transfection procedure using a single pulse at 210 V/cm and 960 μF in PBS. The pLC-2 plasmid (9.1 kb) was obtained by introducing the lacZ gene (HindIII–BamHI) from pCH110 (Pharmacia), encoding for the bacterial β-galactosidase, at the polycloning site of the pRE/CMV construct (5.4 kb; Invitrogen) adjacent to the HCMV IE1–2 promoter/enhancer region. Transfected cells were selected and cloned in RPMI-1640 medium with G-418 sulphate at 1 mg/ml. The selected clone used in these experiments, was characterized by a low, constitutive β-galactosidase expression and a high phorbol-12-myristate-13-acetate (PMA; Sigma) and phyt-oheamaglutinin (PHA; Wellcome) inducibility (5–10-fold).

4-Methylumbelliferyl β-D-galactoside (MUG) assay for β-galactosidase activity. For assays, pCMV-LacZ Jurkat T cells in the exponential growth phase were plated in 96-well Costar microtitre plates (10⁴ cells per well in 200 μl of medium). The expression of β-galactosidase was monitored using the MUG assay, performed according to the method of Roederer et al. (1990) with some modifications, as described below.

Cells were lysed by adding 20 μl of 0.5% Triton X-100 in Z buffer (60 mM-Na₂HPO₄, 40 mM-NaH₂PO₄, 10 mM-KCl, 1 mM-MgSO₄ pH 7.0) to each well, followed by freezing and thawing.

MUG (Sigma) in Z buffer (diluted 10-fold from 15 mM stock in dimethylformamide) was added to a final concentration of 0.6 mM (80 ml). The incubations were carried out for 30 min at room temperature. Reactions were stopped by adding 100 μl of Stop buffer (15 mM-EDTA, 300 mM-glycine, pH 11.2). Fluorescence was determined using the Millipore CytoFluor 2300 fluorescence plate reader with an excitation at 360 nm and an emission at 460 nm. Untransfected Jurkat T cells were plated and treated in the same way as the pCMV-LacZ cell line to evaluate the possible effects of drugs on the endogenous cellular β-galactosidase activity. In all experiments, the emission value of MUG in culture medium was subtracted from the experimental values. Results are expressed in UFGal (fluorescence units of galactosidase) per 10⁴ viable cells.

Colorimetric assays for reduced glutathione. Plasmid pCMV-LacZ Jurkat T cells (2×10⁶) were washed twice with PBS and resuspended in 20 ml PBS and 100 ml 0.01 M HCl to inactivate γ-glutamyltranspeptidase. The cells were lysed by freezing and thawing twice. After removing cell debris (5000 r.p.m. for 5 min at 4 °C) the supernatant solution was deproteinized with 50 ml of 10% S-sulphosalicylic acid. The protein-free solution was stored at 1 mg/ml. The selected clone used in these experiments, was characterized by a low, constitutive β-galactosidase expression and a high phorbol-12-myristate-13-acetate (PMA; Sigma) and phyt-oheamaglutinin (PHA; Wellcome) inducibility (5–10-fold).

The supernatant was removed from the cells by centrifugation at 4 °C. After freezing and thawing, the supernatant was centrifuged at 14,000 r.p.m. for 10 min. The supernatant was removed and the cells were washed two times with PBS and resuspended in 100 ml 0.01 M HCl. After freezing and thawing, the supernatant was centrifuged at 14,000 r.p.m. for 10 min. The supernatant was removed and the cells were washed two times with PBS and resuspended in 100 ml 0.01 M HCl. After freezing and thawing, the supernatant was centrifuged at 14,000 r.p.m. for 10 min. The supernatant was removed and the cells were washed two times with PBS and resuspended in 100 ml 0.01 M HCl.
Results

Replication of HCMV in MRC-5 cells pretreated with VPA

When MRC-5 cells were infected with HCMV at an m.o.i of 10^2 TCID_{50}/ml, CPE appeared 5 days p.i. However, pretreatment of MRC-5 cells with 2.5 to 10 mM-VPA for 24 h before infection, markedly enhanced the CPE produced by HCMV. In 10 mM-VPA pretreated cells, HCMV-specific CPE appeared as early as 3 days p.i. and approximately 70% of the cells had CPE at 5 days p.i. The viral yield, determined 7 days p.i., increased in the presence of VPA, in a dose-dependent manner (Table 1). When cells were pretreated with 10 mM-VPA, 1000- to 2000-fold more virus was produced. When cells were treated with VPA immediately after virus adsorption, the effect on HCMV replication was significantly smaller (data not shown). In a similar manner, we observed that VPA did not enhance replication of herpes simplex virus type 1 (HSV-1) or of varicella-zoster virus (VZV) in MRC-5 cells (data not shown).

Effect of VPA on IE and E HCMV functions

After migration of viral DNA to the host cell nucleus, the first event leading to a properly programmed productive cycle requires expression of viral IE functions (Stinski, 1990). To clarify the mechanism by which VPA increased HCMV replication in MRC-5 cells, we examined the effect of VPA on expression of HCMV IE and E functions. An indirect immunoperoxidase assay using MAbs E13 and MAbs 2A2, which recognize the HCMV IE1-2 and E gene products, revealed that the amount of IE and E antigen increased, in a dose-dependent manner, in cells treated with VPA for 24 h before infection (Table 2). When cells were treated with 10 mM-VPA, synthesis of IE antigen 24 h p.i. was 10-fold higher than in untreated cells (Table 2). There also were 24-fold more cells expressing E nuclear antigen 48 h p.i. Treatment with VPA for 24 h before infection was required to observe an effect. Indeed, when cells were treated with

<p>| Table 1. Effect of various concentrations of VPA on replication of HCMV in MRC-5 cells* |
|----------------------------------------|--------|--------|</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Virus yield (TCID_{50}/ml)</th>
<th>Fold induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>2.9 × 10^4</td>
<td>1</td>
</tr>
<tr>
<td>2.5 mM-VPA</td>
<td>7.10^4</td>
<td>24</td>
</tr>
<tr>
<td>5.0 mM-VPA</td>
<td>1.9 × 10^4</td>
<td>660</td>
</tr>
<tr>
<td>10.0 mM-VPA</td>
<td>4.1 × 10^4</td>
<td>1400</td>
</tr>
</tbody>
</table>

* Cells were treated with VPA within 24 h before HCMV infection with 10^7 TCID_{50}/ml. The virus yield was measured 7 days post-infection.

Table 2. Effect of VPA on production of IE and E antigens in MRC-5 cells infected with HCMV*

<table>
<thead>
<tr>
<th>VPA (mM)</th>
<th>IE antigen-positive cells/well</th>
<th>Fold increase</th>
<th>E antigen-positive cells/well</th>
<th>Fold increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>12</td>
<td>1.00</td>
<td>6</td>
<td>1.00</td>
</tr>
<tr>
<td>1.25</td>
<td>16</td>
<td>1.33</td>
<td>9</td>
<td>1.50</td>
</tr>
<tr>
<td>2.5</td>
<td>42</td>
<td>3.50</td>
<td>19</td>
<td>3.17</td>
</tr>
<tr>
<td>5</td>
<td>81</td>
<td>6.75</td>
<td>52</td>
<td>8.67</td>
</tr>
<tr>
<td>10</td>
<td>112</td>
<td>9.33</td>
<td>146</td>
<td>24.33</td>
</tr>
</tbody>
</table>

* IE and E antigens were detected respectively 24 h and 48 h post-infection. Results are expressed in number of IE or E antigen-positive cells/well of a microtitre plate. They are the mean of quadruplicates and are representative of three independent experiments.

Table 3. Effect of VPA on HCMV IE1−2 promoter directed IEA expression in MRC-5 cells*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IE antigen-positive cells/well</th>
<th>Fold increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>21</td>
<td>1.00</td>
</tr>
<tr>
<td>1.25 mM-VPA</td>
<td>35</td>
<td>1.60</td>
</tr>
<tr>
<td>2.5 mM-VPA</td>
<td>102</td>
<td>4.80</td>
</tr>
<tr>
<td>5 mM-VPA</td>
<td>121</td>
<td>5.70</td>
</tr>
<tr>
<td>10 mM-VPA</td>
<td>170</td>
<td>8.00</td>
</tr>
</tbody>
</table>

* Cells were treated with VPA 24 h post-transfection with pRR47 and IE antigens were detected 24 h post-treatment.

VPA immediately after HCMV adsorption, there was no increase in the IE and E antigen inclusions (Table 2). In addition, the increase in the number of IE and E antigen-positive cells after treatment with VPA, also depended on the infectious dose used. The effect was higher when low doses of virus were used (data not shown).

Effect of VPA on HCMV IE1−2 promoter-directed IEA expression in MRC-5 cells

To confirm that virus replication increased through an activation of the IE antigen expression, we examined the effect of VPA on the HCMV promoter-directed expression of IE antigen using a plasmid (pRR47) carrying the entire IE1−2 gene region. Twenty-four hours after transfection, MRC-5 cells were treated with various doses of VPA. The number of IE antigen-positive cells was determined 24 h after treatment (48 h after transfection) (Table 3). In untreated samples, 21 cells expressing IEA were counted per well 48 h post-transfection. If cells were treated with 1.25 to 10 mM-VPA immediately after HCMV adsorption, there was no increase in the IE and E antigen inclusions (Table 2). In addition, the increase in the number of IE and E antigen-positive cells after treatment with VPA, also depended on the infectious dose used. The effect was higher when low doses of virus were used (data not shown).
VPA, this number increased, in a dose-dependent manner, up to 170 IE antigen-positive cells per well, i.e. 8-fold more than without treatment. VPA was added 24 h after transfection and therefore acts at the level of IE antigen expression.

**Effect of VPA on pCMV-LacZ Jurkat T cell line induction**

Even if T cells are not a productive site for HCMV, they may be a reservoir for maintaining natural infection (Rice et al., 1984; Schrier et al., 1985; Braun & Reiser, 1986). To study the effect of VPA on the HCMV promoter, we constructed a stably transfected Jurkat T cell line containing the bacterial lacZ reporter gene under the control of the IE1–2 HCMV promoter/enhancer. In this cell line, β-galactosidase is inducible by treatment with PMA and PHA. We first confirmed that VPA induced transactivation of HCMV promoter. β-Galactosidase activity measured after VPA treatment increased in a dose-dependent manner in the range of 2.5 to 10 mM. For example, after 24 h of a 10 mM-VPA treatment, β-galactosidase activity was around 7- to 10-fold higher than in untreated cells (Fig. 1). This induction of transcription by VPA began only after 10 h of treatment. The maximum level of stimulation by VPA occurred after 30 h of treatment, in contrast to PMA and PHA which stimulated within 8 to 10 h (Fig. 2).

We then studied the effect of VPA on PMA or PHA-mediated HCMV promoter transactivation. First, we treated the pCMV-LacZ Jurkat T cells with PMA or PHA and with 5 mM-VPA simultaneously. We observed that the β-galactosidase expression mediated by PMA or PHA was inhibited by 40 to 60% by VPA treatment, 4 h post-induction (Fig. 3). However, after a longer period of treatment with VPA, the maximum level of induction by PMA or PHA was increased and reached not within 8 to 10 h post-induction, but later. In the experiment reported, at 22 h post-induction, the PMA and PHA effects were respectively 2.6- and 1.66-fold higher in the presence of VPA than in mock-treated cells.

If the cells were pretreated with VPA within 18 h before induction with PMA or PHA, these two inducers immediately provided a strong activation of the HCMV
HCMV activation by sodium valproate

Fig. 4. Effect of a VPA (5 mM) pretreatment (18 h before induction) on PMA (20 ng/ml) and PHA (2 µg/ml)-mediated transactivation of the HCMV promoter in the pCMV-LacZ Jurkat T cell line. Symbols: □, PMA; ■, PMA+VPA; △, PHA; ▲, PHA+VPA; ○, VPA.

promoter (Fig. 4). In the case of PMA, the β-galactosidase activity measured 24 h post-induction was 50-fold higher when cells had been pretreated with 5 mM-VPA. In the case of PHA, the effect was less spectacular, but reached the 7- to 10-fold range.

We verified that the effect of VPA was specific for an activation of the HCMV promoter, since VPA did not have any effect on the endogenous β-galactosidase activity in Jurkat T cells (data not shown). Moreover, measurement of thymidine incorporation showed that VPA treatment did not stimulate cellular replication (data not shown).

Effect of VPA on intracellular GSH levels

In order to explain the mechanism of action of VPA on HCMV promoter transactivation and on HCMV replication, we compared the intracellular GSH levels, before and after VPA treatment, in Jurkat and MRC-5 cells. GSH quantification showed that VPA treatment led to a decrease, in a dose-dependent manner, of the level of this thiol in Jurkat T cells but not in MRC-5 cells (Table 4). Measurements were performed over various periods of time after treatment. The GSH level was reduced by 60 to 80% after 24 h of treatment with VPA in Jurkat T cells. On the contrary, VPA did not decrease the level of this thiol in MRC-5 cells. Buthionine-S,R-sulphoximine (BSO; 10 mM), an irreversible inhibitor of γ-glutamylcysteine synthetase, was used as a control.

Discussion

In this report, we have shown that valproic acid, an anticonvulsant drug, frequently prescribed as a therapeutic agent for the management of many forms of epilepsy (Chapman et al., 1982), was able to stimulate HCMV replication in human fibroblasts in a dose-dependent manner. Moreover, a pretreatment of cells was necessary to obtain maximum levels of stimulation. We observed that the enhancement of virus replication correlated with an increase of the number of IE and E antigen-positive cells. When cells were transfected with a plasmid containing the entire IE1–2 region, we noted that VPA also induced the expression of IE antigens. Even if an effect of VPA on the earliest events of the infectious process, such as virus adsorption, penetration and uncoating of HCMV cannot be ruled out, an effect on IE antigen expression can explain the stimulation of viral replication. Furthermore, we have shown that the induction of IE gene expression occurred through activation of the promoter/enhancer sequences of the IE1–2 region, using a Jurkat T cell line stably transfected with a construct containing a reporter gene under the control of the HCMV promoter/enhancer.

Table 4. Effect of VPA on the GSH level in Jurkat T cells and in MRC-5 cells*

<table>
<thead>
<tr>
<th>Time post-treatment (h)</th>
<th>Untreated cells nmol/10⁶ cells</th>
<th>10 mM-VPA nmol/10⁶ cells</th>
<th>10 mM-BSO† nmol/10⁶ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jurkat T cells</td>
<td></td>
<td>T/UT†</td>
<td>T/UT</td>
</tr>
<tr>
<td>3</td>
<td>2.9</td>
<td>3.0</td>
<td>3.3</td>
</tr>
<tr>
<td>6</td>
<td>2.4</td>
<td>1.4</td>
<td>0.8</td>
</tr>
<tr>
<td>22</td>
<td>3.4</td>
<td>0.8</td>
<td>0.02</td>
</tr>
<tr>
<td>MRC-5 cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>106 103</td>
<td></td>
<td>38 38</td>
</tr>
</tbody>
</table>

* GSH assays were monitored 3, 6, 22 or 24 h post-treatment.
† T/UT (Treated/Untreated) is percentage of variation with respect to the control.
‡ BSO (buthionine-S,R-sulphoximine) is an irreversible inhibitor of γ-glutamylcysteine synthetase, and was used as a positive control. Results are representative of four independent experiments.
Several mechanisms to explain the VPA effect on HCMV replication must be considered. We have investigated whether VPA could act through the GSH metabolic pathway. Indeed, VPA in association with carbamazepine is known to decrease the cerebral levels of glutathione (Attagui et al., 1992) and to inhibit glutathione reductase (GR) in the liver (Cotariu et al., 1990) and in the red blood cells (Cotariu et al., 1992). We surmised that VPA, by reducing GSH intracellular level, which is the major cellular antioxidant (Meister, 1991), could indirectly prevent cells from being protected from reactive oxygen species (ROS) which are produced during metabolism and T cell activation (Fulbert & Cals, 1992). These ROS were described to be involved in activation of the transcription factor NF-κB (Schreck et al., 1991), which could result in IE1–2 promoter transactivation and viral replication (Sambucetti et al., 1989). We actually observed a significant decrease in the GSH level in VPA-treated Jurkat T cells. However, in human fibroblasts, VPA did not modify the GSH level. Moreover, when the GSH synthesis pathway was blocked with BSO, which decreased the intracellular GSH level by 90 to 100%, we only observed a slight enhancement of HCMV promoter transactivation, but never observed any effect on HCMV replication (data not shown). Therefore, we cannot explain the effect of VPA by an action on GSH metabolism, notably in MRC-5 cells. In addition, Nordhoff et al. (1994) recently reported that they could not detect any significant effect of VPA on glutathione reductase.

Since activation of the HCMV IE1–2 promoter regulatory region is mainly dependent on host cellular factors (Ghazal et al., 1987, 1988), it is possible that one or more such cellular protein(s) were activated or synthesized when cells were pretreated with VPA. However, as shown by the time course of action on β-galactosidase expression in the pCMV-LacZ Jurkat T cell line, the mechanism involved is different from that of PMA and PHA, i.e. activation of protein kinase C which could induce NF-κB activity (Nabel & Baltimore, 1987; Niller & Hennighausen, 1990).

As the VPA effect did not appear immediately after treatment, VPA may act indirectly through one of its metabolites. Indeed, numerous authors agree that unravelling the molecular basis of its anticonvulsant action (and side effects), requires a comprehensive analysis of its metabolites, some of which possess a significant pharmacological activity. Four metabolic pathways have been found: glucuronidation, β-oxidation, ω-oxidation, (ω-1)-oxidation and the metabolism of valproate appears to be complex in regard to the natural fatty acids normally absorbed in man. Vamecq et al. (1993) reported that the coenzyme A esters of valproic acid and related metabolites are handled by a peroxisomal β-oxidation pathway distinct from fatty acid and bile acid β-oxidation routes. In this way, VPA and related metabolites have the capacity to induce higher cellular hydrogen peroxide (H₂O₂) levels because of their ability to act as substrates for H₂O₂-generating oxidases (Van den Branden & Roels, 1985). Further investigation is necessary to establish whether HCMV replication in VPA-treated cells depends on increased oxidative stress.

VPA being a short-chain fatty acid, we considered a possible analogy with sodium butyrate. Indeed, sodium butyrate, another short-chain fatty acid, unbranched in contrast to VPA, was shown to induce HCMV replication in human endothelial cells (Radsak et al., 1989) and in a human epithelial cell line (Tanaka et al., 1991). This compound had been previously described to induce a variety of morphological and biochemical changes in different cell types (Prasad & Sinha, 1976), and also to stimulate the replication of other herpesviruses, Epstein–Barr virus and herpes simplex virus (Luka et al., 1979; Saemundsen et al., 1980; Ash, 1986) and more recently HIV (Bohan et al., 1989; Laughlin et al., 1993). We had reported that VPA also stimulated HIV replication (Simon et al., 1994). We have observed that sodium butyrate potentiated replication of HCMV in human fibroblasts and activated HCMV promoter transactivation (data not shown). The kinetics of induction of pCMV-LacZ Jurkat T-cell line by sodium butyrate were similar to the kinetics of induction by VPA. Therefore, it is possible that the two compounds acted by the same mechanism. Despite a large number of reports on butyrate’s effects on gene expression, little is known of its mechanism(s) of action. Recent work has suggested that butyrate could act through inhibition of histone deacetylase (Laughlin et al., 1993).

For the time being, it is very difficult to propose a hypothesis relative to the mechanisms by which VPA activates the HCMV promoter and stimulates HCMV replication. However, the ability of VPA to activate HCMV replication described in this paper, provides an attractive model for studying the molecular basis of the regulation of HCMV IE1–2 gene expression by cellular factors and its relationship to HCMV latency and reactivation of latent virus.

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References


