Effect of valproate derivatives on human brain myo-inositol-1-phosphate (MIP) synthase activity and amphetamine-induced rearing

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Abstract:
We have recently shown that valproate (VPA) decreases intracellular concentrations of inositol, like lithium but via a different mechanism, namely by inhibiting myo-inositol-1-phosphate (MIP) synthase. Valnoctamide (VCD) and valrocemide (VGD) are VPA derivatives which are anticonvulsants and have been shown in animal models to be significantly less teratogenic than VPA. We now show that 1 mM of either VCD or VGD drastically inhibits human brain crude homogenate MIP synthase activity. We studied the mechanism of the effect of VCD and found that it reduced the enzyme activity by an apparent competitive mode of inhibition at concentrations within the therapeutic range of VPA (Kᵢ = 0.18 mM). We studied the behavioral effect of VGD and found that both lithium and VGD attenuated amphetamine-induced increase in rearing. These data support clinical study of these VPA-derivatives in bipolar disorder.

Key words:
bipolar disorder, valnoctamide, valrocemide, myo-inositol-1-phosphate (MIP) synthase, teratogenicity, amphetamine

Introduction

Valproic acid (VPA) is an anticonvulsant mood-stabilizer widely used in the treatment of bipolar disorder [8, 22, 26]. Administration of VPA during the first trimester of pregnancy significantly increases the risk of embryo neural tube defects, such as spina bifida, therefore, limits its use in women in childbearing age [3, 14, 15]. Other mood stabilizers such as lithium and carbamazepine are also teratogenic during pregnancy [6, 25] and, therefore, the treatment of young bipolar women is problematic.

We have recently shown that VPA, like lithium, acutely decreases intracellular concentrations of inositol in mouse brain, albeit via a different molecular target [21]. While lithium inhibits the key enzyme of inositol recycling in the phosphatidylinositol signaling pathway, inositol monophosphatase [10], VPA indirectly inhibits the key enzyme of inositol de-novo synthesis from glucose, myo-inositol-1-phosphate (MIP) synthase, both in yeast [23] and in human brain crude homogenates by an unknown mechanism [12, 21].

Valnoctamide (VCD, 2-ethyl-3-methyl valeramide) is a derivative of VPA, which undergoes only minimal
biotransformation to VPA [2, 9, 17]. In mice it has been shown to be significantly less teratogenic and embryolethal than VPA [18]. Animal studies reported that VCD was at least as efficient anticonvulsant as VPA [2, 3, 13].

Valrocemide (N-valproyl glycaminide, VGD or TV-1901), the conjugation product of VPA and glycaminide, has been shown to be a potent antiepileptic drug [1]. VGD did not show teratogenic potential in rats or rabbits [1] or in an inbred mouse strain (SWV) sensitive to antiepileptic drug-induced teratogenicity [11]. VGD administered iv to rats is almost completely eliminated as the inactive acid valproyl-glycine [5], thus acting independently and not as a prodrug of VPA.

The aim of the present study was to identify non-teratogenic VPA derivatives with clinical potential in bipolar disorder. We compared the effect of several VPA derivatives, especially VCD and VGD, on crude postmortem human brain homogenate MIP synthase activity. Since VCD and VGD were found to be most potent on MIP synthase, we studied the mode of inhibition of MIP synthase with one compound, VCD, and the behavioral effect of another compound, VGD, on amphetamine-induced rearing, a well established behavioral effect of therapeutically-relevant lithium concentrations.

Materials and Methods

Experimental Procedures

Human brain

Human brain tissue. Right hemisphere human postmortem prefrontal cortex samples from subjects with no history of psychiatric disorders, derived from a collection previously described [7], were used to measure MIP synthase activity. The use of the human brain collection was approved by the Soroka Hospital Helsinki Committee.

Human brain MIP synthase activity. MIP synthase activity was measured by a modification of the procedures of Wong et al. [24] and Novak et al. [16]. Aliquots (5 μl) of the supernatant fraction obtained after sonication (Ultrasonic Processor, Newtown, CT, 15 s at 0.1 output watts at 4°C) and centrifugation for 20 min at 9,000 × g at 4°C of postmortem human prefrontal cortex (1 mg wet weight in 0.5 ml of 50 mM Tris HCl, pH 7.4) were added to a final volume of 30 μl reaction mixture containing varying concentrations of D-glucose-6-phosphate, 1.25 μCi [14C]D-glucose-6-phosphate 1.6 mM NAD+, 0.45 mM KCl, 5.4 mM MgCl2 and 0.9 mM Tris HCl, pH 7.6, with or without the indicated VCD concentration. VCD was custom-synthesized by Zyfine, Ahmedabad, Gujarat, India. Incubation was carried out for 2.5 h at 37°C (this was determined experimentally to be within the linear range of activity). The reaction was stopped by heating the tubes for 5 min at 90°C. IMPase (10 mU) Sigma, St. Louis) or IMPase buffer (150 mM KCl, 50 mM Tris HCl, 0.1 mM EGTA, 0.5 mM EDTA, pH 8.5) were added to a final volume of 40 μl and a second incubation was run for 1 h at 37°C. The reaction was stopped by adding 40 μl of cold double distilled water (ddH2O). Seventy out of the 80 μl were added to test tubes containing 1.25 g of strong basic anion resin (Amberjet 4200, Rohm and Haas, Philadelphia) in 1 ml of ddH2O. The mixtures were vortexed for 10 min, then centrifuged for 10 min at 4,000 × g at room temperature, and 100 μl of supernatant was taken for 14C counting (Liquid Scintillation β-Counter, Kontron, Basel). The enzymatic activity was calculated by subtracting the values obtained with IMPase minus values obtained without it. The use of excess IMPase ensures that the decrement represents only the product of MIP synthase. The measurements were carried out in triplicate. The drugs were dissolved in 12.5% ethanol. Final ethanol concentration in the assay (both in the control and in the drug effect assays) was 2.1%.

Animals

The study was approved by the Institutional Committee for the use of animals in research.

Administration of VGD in food. Forty male Sprague-Dawley rats weighing 200–250 g were divided into four groups and housed in groups of 3–4/cage under a 12 h light/dark schedule and ambient temperature of 23°C. All behavioral tests were conducted in the light phase. Drug pretreatment was: group 1 – powdered chow, group 2 – 2 g/kg lithium in pellets, as a positive control, resulting in serum lithium levels of 0.88 ± 0.14 mM, group 3 – 100 mg/kg VGD (Teva, Jerusalem, Israel) and group 4 – 200 mg/kg VGD. Administration of the drug in food better resembles...
chronic oral drug treatment in humans and is a well established method to reach therapeutically relevant blood concentrations of drugs in animal models [20].

Rearing was tested in an automated activity monitor (Elvicom, Israel) for 30 min 2.5 weeks after initiation of diet. The testing took place on 2 days (20 rats/day). On the first day half of the rats from each group were given 0.5 mg/kg amphetamine subcutaneously while the other half received saline. The following day the rats that had received saline received amphetamine and vice versa. Thus, the order was balanced. A within subject, repeated measure design was used to test the effect of saline and amphetamine injections on rearing.

Results

Postmortem human brain mip synthase activity

Effect of VPA derivatives on MIP synthase activity.
Table 1 shows that all VPA derivatives tested had an effect on postmortem human brain crude homogenate MIP synthase activity. VCD or VGD (1 mM) abolished the activity. We, therefore, studied these compounds more extensively, one biochemically and one behaviorally.

Characterization of the effect of VCD on MIP synthase activity. Figure 1 shows that human brain MIP synthase activity is reduced by VCD within the therapeutically relevant range of concentrations of VPA, with an IC$_{50}$ ~ 0.55 mM. MIP synthase activity was further measured at substrate (glucose-6-phosphate) concentrations in the range of 0.5–5.0 mM in the presence or absence of 0.6 mM VCD. The Lineweaver-Burk plot (Fig. 2) revealed an apparent competitive mode of inhibition of MIP synthase by VCD. The kinetic parameters (apparent K$_m$ and V$_{max}$) in the absence and in the presence of 0.6 mM VCD are summarized in Table 2. The apparent K$_i$ for the effect of VCD on MIP synthase derived from the Lineweaver-Burk plot is 0.18 mM.

Amphetamine-induced rearing in rats

Effect of VGD. VGD (100 mg/kg) administration attenuated amphetamine-induced rearing activity compared to control rats, as did lithium, the classic anti-

Effect of VPA derivatives on MIP synthase activity.

Tab. 1. VPA-derivatives affect human brain crude homogenate MIP synthase activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (mM)</th>
<th>Reduction of human brain MIP synthase activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Newly reported</td>
<td>VTD</td>
<td>1.0</td>
</tr>
<tr>
<td>VCD</td>
<td>1.0</td>
<td>96</td>
</tr>
<tr>
<td>VGD</td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td>Previously reported* VPA</td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td>VPD</td>
<td>1.4</td>
<td>34</td>
</tr>
<tr>
<td>M-TMCD</td>
<td>1.3</td>
<td>93</td>
</tr>
</tbody>
</table>

MIP synthase activity was measured as described in Materials and Methods in the presence and in the absence of each of the drugs at the given concentration, n = 5 control, 2 valproyl valpropamide (VTD), 4 VCD, 4 VGD, 10 VPA, 4 valproamide (VPD), 2 N-methyl D,L-aspartate (VPA), 10 valproicamide (M-TMCD). The drugs (kindly donated by Prof. Meir Edel, School of Pharmacy, Hebrew University, Jerusalem, Israel) were dissolved in 12.5% ethanol. Final ethanol concentration in the assay (both in the control and in the drug effect assay) was 2.1%. * [21]
The aim of the present study was to identify non-teratogenic VPA derivatives with clinical potential in
bipolar disorder. We show that at 1 mM VCD and VGD drastically inhibited postmortem human brain crude homogenate MIP synthase activity. We characterized the mode of the effect of VCD on MIP synthase activity in human prefrontal cortex. An apparent competitive mode of inhibition of brain MIP synthase activity by VCD was found, differently than was previously reported by us for VPA, that affects MIP synthase activity by a non-competitive manner [21]. The amide group present only in VCD may alter the mode of interaction of the drug with the enzyme as is the case of the inhibition of acetylcholinesterase by hydroxyphenyl-trimethylammoniumiodide, that competitively inhibits the enzyme, vs. its nitrophenyl-sulfonoxyl derivative, that noncompetitively inhibits it [19]. The $K_i$ for the indirect inhibition of crude homogenate MIP synthase by VPA was 0.21–0.28 mM [21], and was comparable with the $K_i$ now found for VCD, 0.18–0.55 mM derived from the Lineweaver-Burk plot (Fig. 2) and the activity as a function of the inhibitor’s concentration (Fig. 1).

**Discussion**

The aim of the present study was to identify non-teratogenic VPA derivatives with clinical potential in
We chose to study the behavioral effect of VGD, the other compound that achieved maximal inhibition of MIP synthase. Attenuation of amphetamine-induced rearing is a well established behavioral effect of therapeutically relevant lithium concentrations. Similarly, VGD administration to rats (100 mg/kg in food) attenuated their rearing activity in response to amphetamine as in the case of lithium-treated rats compared to control rats. However, the higher concentration of VGD was ineffective and actually tended to increase activity, suggesting a narrow window of effective anti-manic concentration. It is possible that the parallel effect of lithium and VGD is mediated by a common effect of inositol depletion, with lithium inhibiting inositol monophosphatase and VGD, like VPA, decreasing MIP synthase activity.

The possibility that the two non-teratogenic VPA-derivatives, VCD and VGD, are potential drugs for study in bipolar disorder awaits further investigation in clinical trials. Both VCD and VGD were patented as potential mood stabilizers (US6417399 and US-5585358, respectively).

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References:


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