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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 (K_i = 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 glycinamide, VGD or TV-1901), the conjugation product of VPA and glycinamide, 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 [^{14}C]D-glucose-6-phosphate 1.6 mM NAD^+, 0.45 mM KCl, 5.4 mM $MgCl_2$ 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 (ddH_2O). 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 ddH_2O. 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 ^{14}C 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 antimanic treatment. Two-way ANOVA revealed a significant effect of pretreatment ($F = 7.46$, $p < 0.001$), a significant effect of amphetamine ($F = 40.5$, $p < 0.001$) and a significant interaction between the pretreatment and amphetamine ($F = 3.32$, $p = 0.03$). To further analyze the data, the saline and amphetamine conditions were analyzed separately with post-hoc Neuman Keuls tests used due to abnormal distribution. There was a significant effect of the drug on saline-injected rats ($F = 3.78$, $p < 0.02$) and the post-

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

	Treatment	Concentration (mM)	Reduction of human brain MIP synthase activity (%)
	Control		0
Newly reported	VTD	1.0	55
	VCD	1.0	96
	VGD	1.0	100
Previously reported*	VPA	1.0	100
	VPD	1.4	34
	M-TMCD	1.3	93

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 taurinamide (VTD), 4 VCD, 4 VGD, 10 VPA, 4 valpromide (VPD), 2 N-methyl-2,2,3,3,-tetramethyl-cyclopropane carboxamide (M-TMCD). The drugs (kindly donated by Prof. Meir Bialer, 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 assays) was 2.1%. * [21]

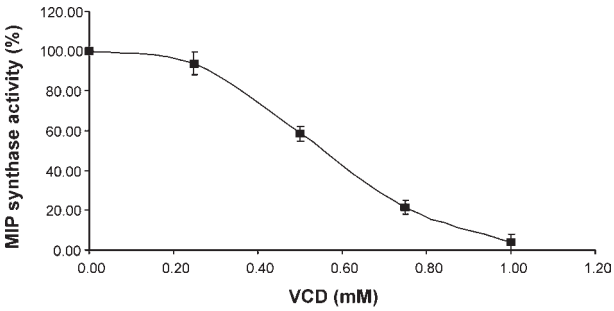

Fig. 1. Human brain MIP synthase activity is reduced by VCD within the therapeutically-relevant range of concentrations of VPA. Results are means ± SEM; n = 6 (0 mM), 2 (0.25 mM), 3 (0.5 mM), 2 (0.75 mM) and 6 (1 mM)

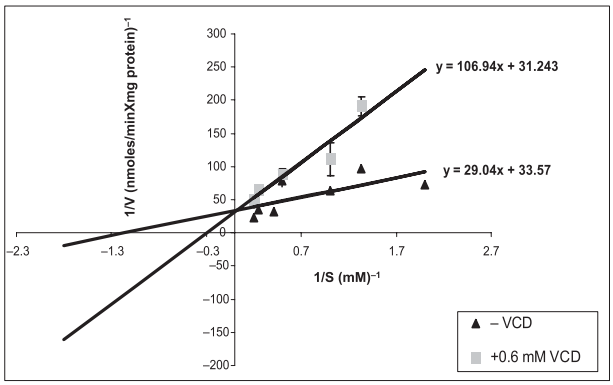

Fig. 2. VCD reduces MIP synthase activity by an apparent competitive mode of inhibition (a Lineweaver-Burk plot). The intersect of the best fit line with the X axis = –1/apparent K_m. The intersect of the best fit line with the Y axis = 1/apparent V_{max}. Results are means ± SEM; n = 2–5 measurements for each point

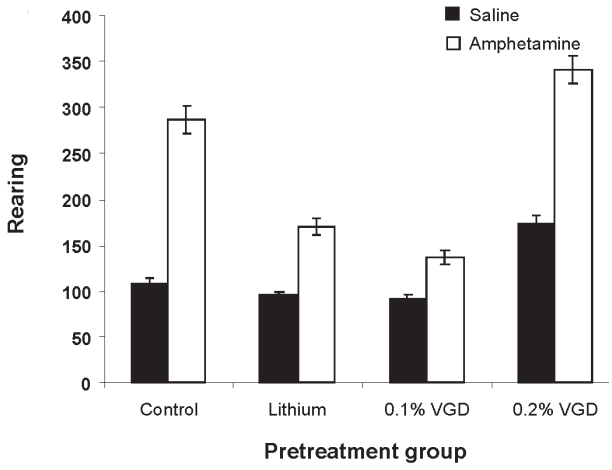

Fig. 3. Effect of VGD on amphetamine-induced rearing in rats. The Y axis represents the number of beam breaks on the automated monitor. Results are means ± SEM; n = 10 for each treatment. Two-way ANOVA revealed a significant effect of pretreatment ($F = 7.46$, $p < 0.001$), a significant effect of amphetamine ($F = 40.5$, $p < 0.001$) and a significant interaction between the pretreatment and amphetamine ($F = 3.32$, $p = 0.03$). There was a significant effect of the drug in saline-injected rats ($F = 3.78$, $p < 0.02$) and the *post-hoc* Newman Keuls comparisons indicated that 200 mg/kg VGD significantly elevated rearing compared to each of the other groups ($p < 0.03$). There was also a significant effect of pretreatment on amphetamine-induced hyperactivity ($F = 6.52$, $p = 0.001$). *Post-hoc* test indicated that 200 mg/kg VGD had significantly greater rearing activity than the groups treated with lithium or 100 mg/kg VGD

Tab. 2. Apparent K_m and V_{max} of MIP synthase in the absence and in the presence of 0.6 mM VCD

0.6 mM VCD	K_m (mM)	V_{max} (nmoles/minXmg protein)
without	0.8	0.031
with	3.4	0.031

K_m and V_{max} values are derived from the Lineweaver-Burk plot (Fig. 2)

hoc comparisons indicated that 200 mg/kg VGD significantly elevated rearing compared to each of the other groups ($p < 0.03$). There was also a significant effect of pretreatment on amphetamine induced hyperactivity ($F = 6.52$, $p = 0.001$). *Post-hoc* test indicated that 200 mg/kg VGD had significantly greater rearing activity than the groups treated with lithium or 100 mg/kg VGD. The lithium- and 100 mg/kg VGD-pretreated groups had significantly lower rearing activity than control rats and than those treated with 200 mg/kg VGD (Fig. 3).

Discussion

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).

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:

1. Anderson GD, Acheampong, AA, Wilensky AJ, Levy RH: Effect of valproate dose on formation of hepatotoxic metabolites. Epilepsia, 1992, 33, 736–742.
2. Bialer M: Clinical pharmacology of valpromide. Clin Pharmacokinet, 1991, 20, 114–122.
3. Bialer M, Haj-Yehia A, Badir K, Hadad S: Can we develop improved derivatives of valproic acid? Pharm World Sci, 1994, 16, 2–6.
4. Bialer M, Haj-Yehia A, Barzaghi N, Pisani F, Perucca E: Pharmacokinetics of a valpromide isomer, valnoctamide, in healthy subjects. Eur J Clin Pharmacol, 1990, 38, 289–291.
5. Blotnik S, Bergman F, Bialer M: The disposition of valporyl glycinamide and valproyl glycine in rats. Pharm Res, 1997, 14, 873–878.
6. Gagliardi JP, Krishnan KR: Evidence-based mental health use of anticonvulsants during pregnancy. Psychopharmacol Bull, 2003, 37, 59–66.
7. Gross-Isseroff R, Salama D, Israeli M, Biegon A:. Autoradiographic analysis of [3H]ketanserin binding in the human brain postmortem: effect of suicide. Brain Res, 1990, 507, 208–215.
8. Guay DR: The emerging role of valproate in bipolar disorder and other psychiatric disorders. Pharmacotherapy, 1995, 15, 631–647.
9. Haj-Yehiya A, Bialer M: Pharmacokinetics of a valpromide isomer, valnoctamide, in dogs. J Pharm Sci, 1988, 77, 831–834.
10. Hallcher LM, Sherman WR: The effects of lithium ion and other agents on the activity of myo-inositol-1-phosphatase from bovine brain. J Biol Chem, 1980, 255, 10896–10901.
11. Isoherranen N, Woodhead JH, White HS, Bialer M: Anticonvulsant profile of valrocemide (TV1901): a new antiepileptic drug. Epilepsia, 2001, 42, 831–836.
12. Ju S, Shaltiel G, Shamir A, Agam G, Greenberg ML: Human 1-D-myo-inositol-3-phosphate synthase is functional in yeast. J Biol Chem, 2004, 279, 21759–21765.
13. Loscher W, Nau H: Pharmacological evaluation of various metabolites and analogues of valproic acid. Anticonvulsant and toxic potencies in mice. Neuropharmacology, 1985, 24, 427–435.
14. Nau H, Hauck RS, Ehlers K: Valproic acid-induced neural tube defects in mouse and human: aspects of chirality, alternative drug development, pharmacokinetics and possible mechanisms. Pharmacol Toxicol, 1991, 69, 310–321.
15. Nau H, Headrick X: Valproic acid teratogenesis. ISI Atlas Sci Pharmacol, 1987, 1, 52–56.
16. Novak JE, Turner RS, Agranoff BW, Fisher SK: Differentiated human NT2-N neurons possess a high intracellular content of myo-inositol. J Neurochem, 1999, 72, 1431–1440.
17. Pisani F, Haj-Yehia A, Fazio A, Artesi C, Oteri G, Perucca E, Kroetz DL et al.: Carbamazepine-valnoctamide interaction in epileptic patients: in vitro/in vivo correlation. Epilepsia, 1993, 34, 954–959.
18. Radatz M, Ehlers K, Yagen B, Bialer M, Nau H: Valnoctamide, valpromide and valnoctic acid are much less teratogenic in mice than valproic acid. Epilepsy Res, 1998, 30, 41–48.
19. Savle PS, Medhekar RA, Kelley EL, May JG, Watkins SF, Fronczek FR, Quinn D M, Gandour RD: Change in the mode of inhibition of acetylcholinesterase by (4-nitrophenyl)sulfonoxyl derivatives of conformationally constrained choline analogues. Chem Res Toxicol, 1998, 11, 19–25.
20. Shaldubina A, Einat H, Szechtman H, Shimon H, Belmaker RH: Preliminary evaluation of oral anticonvulsant treatment in the quinpirole model of bipolar disorder. J Neural Transm, 2002, 109, 433–440.
21. Shaltiel G, Shamir A, Shapiro J, Ding D, Dalton E, Bialer M, Harwood AJ et al.: Valproate decreases inositol biosynthesis. Biol Psychiatry, 2004, 56, 868–874.
22. Tohen M, Grundy S: Management of acute mania. J Clin Psychiatry, 1999, 60, Suppl 5, 31–34; Discussion 35–36.
23. Vaden DL, Ding D, Peterson B, Greenberg ML: Lithium and valproate decrease inositol mass and increase expression of the yeast INO1 and INO2 genes for inositol biosynthesis. J Biol Chem, 2001, 276, 15466–15471.

24. Wong YH, Kalmbach SJ, Hartman BK, Sherman WR: Immunohistochemical staining and enzyme activity measurements show myo-inositol-1-phosphate synthase to be localized in the vasculature of brain. J Neurochem, 1987, 48, 1434–1442.
25. Yonkers KA, Wisner KL, Stowe Z, Leibenluft E, Cohen L, Miller L, Manber R et al.: Management of bipolar disorder during pregnancy and the postpartum period. Am J Psychiatry, 2004, 161, 608–620.
26. Zaremba PD, Białek M, Błaszczyk B, Cioczek P, Czuczwar SJ: Non-epilepsy uses of antiepileptic drugs. Pharmacol Rep, 2006, 58, 1–12.

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