

The Dual Nature of the Antiepileptic Drug Valproic Acid, with Possible Beneficial Effects in Alzheimer's Disease

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Abstract

Valproic acid (VPA) is a short fatty acid with strong anticonvulsant properties. It has diverse effects in different tissues with opposing mechanisms of physiological action. Due to the effects on energy, fatty acid, and cholesterol metabolism, it may be a risk factor for the development of diabetes with its associated complications of atherosclerosis, weight gain, hypertension, insulin resistance and other complications. Its negative effects on the endocrine system can have severe health consequences, especially in the female population. VPA produces proinflammatory and proapoptotic effects in the liver and anti-inflammatory and antiapoptotic effects in the central nervous system. It also causes abnormalities in lipid and cholesterol transport in the liver and the reproductive organs, while in neural stem cells it decreases cholesterol accumulation and helps neural growth and differentiation. However, in the CNS it has some beneficial effects, which are proposed to be important in Alzheimer's disease (AD). In AD mouse models, VPA exerted antiapoptotic effects and the expression of transcription factors that promote neurite growth. Most of the adverse pathogenic actions or beneficial molecular effects are not fully understood. We present an overview and comparison of the different properties of VPA and their effects on estrogen and cholesterol metabolism, lipid transport, Alzheimer's disease, and on the physiology of the liver, reproductive organs, and neurons from in vitro and in vivo (in animal models and patients) studies.

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Introduction

Valproic acid (VPA) is a short, branched-chain fatty acid with anticonvulsant properties and one of the most widely prescribed drugs for many

types of epilepsy in children and adults. Typically, it is applied as the sodium salt form, the sodium valproate and VPA, or mixture of both compounds. The therapeutic concentration varies between 50 and 150mg/l in the serum (1-

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Table 1. The results of literature survey on two search engines using the key word Valproate.

Search engine	Total No. of references (1971-2017)	Year 2017 (first trimester)	Year 2016	Year 2015	Year 2014	Year 2013	Year 2012
SCOPUS	15050	86	437	519	596	598	626
PUBMED	17146	203	782	747	805	788	762

3). The LC-MS/MS or chemiluminescent microparticle immunoassay are used for determination of the VPA level in plasma (4). At serum concentrations 200mg/l and above, VPA can have adverse drug reactions, and it is necessary to lower the dosage of the drug to avoid the acute overdose toxic effects that may include hyperthermia/hypothermia, tachycardia, hypotension (with severe overdose), serious respiratory depression necessitating airway assistance, and cardiac arrest (with severe overdose). The central nervous system (CNS) findings in cases of VPA overdose may include: coma, confusion, somnolence, worsened seizure control, dizziness, hallucinations, irritability, headache, ataxia, and cerebral edema. Death by overdose and death associated with chronic complications in adults are recorded as well as death among children (1, 5).

Although VPA is very efficient in treating epilepsy, its chronic therapeutic application is associated with many unwanted adverse drug reactions in certain prone patients (6). Adverse drug reactions, which are described within this manuscript from cited literature sources, include: weight gain, atherosclerosis, hypertension, nonalcoholic fatty liver disease (NAFLD), alterations of adipocytokine homeostasis, increased oxidative stress, insulin resistance, severe liver toxicity, death, abnormalities in estrogen and androgen metabolism, and PCOS (polycystic ovary syndrome) (Figure 1).

On the other hand, experimental data, which are described in manuscripts from cited literature

sources, suggest that VPA also has effects which seem to be beneficial regarding the physiology of neural tissue (Figure 2), especially in Alzheimer's disease. The effects of VPA in the CNS seem to be completely opposite of those occurring in the liver and other tissues.

By survey of the literature in Table 1, major search engines yield between 15000 and 17000 hits when using the key word Valproate. In the last 6 years, there have been 2862 (Scopus) and 4087 (PubMed) published papers on the subject of VPA (Table 1).

This review has been written to summarize the latest evidence of VPA's effects in living organisms. Novel physiological roles are being discovered, which we point to in the last chapter of the review.

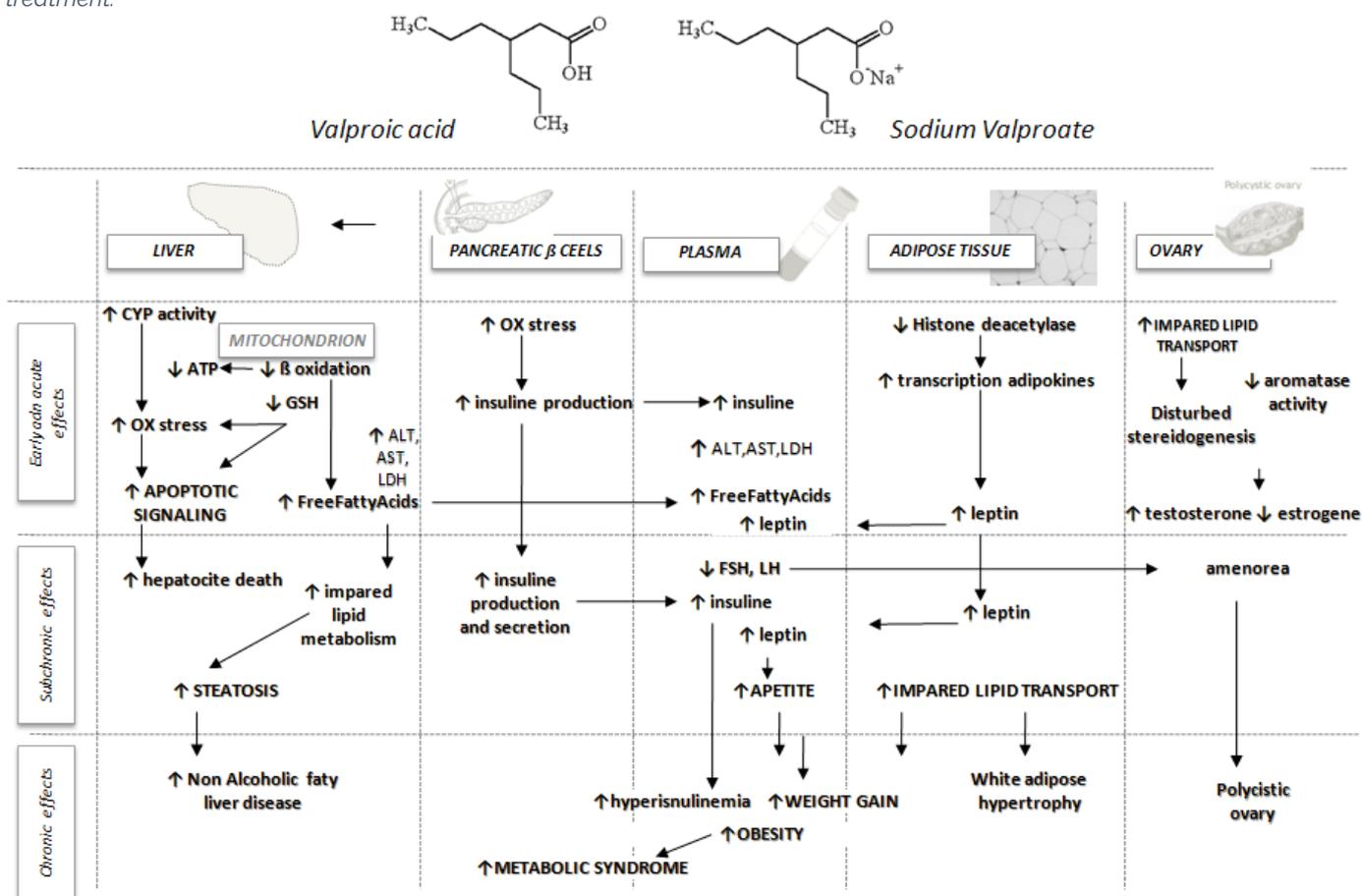
Metabolism, physiological and molecular effects

Absorption, distribution, metabolism and elimination (ADME) of Valproic acid

Pharmacokinetic and pharmacodynamic studies show that the absorption of short fatty acid VPA in the gastrointestinal system is close to 100 % (7,8). Because the molecular structure of VPA is similar to other short and long chain fatty acids, it follows the absorption pathways of all other triglycerides, cholesterol, and fatty acids. Nearly 90% of VPA is bound to the serum proteins. Only the unbound portion of the compound is active. The biological halftime of elimination in patients is 6-16 hours (1). The time of elimination is extended in children younger than 18 months of age. In the organism, there are at least 50

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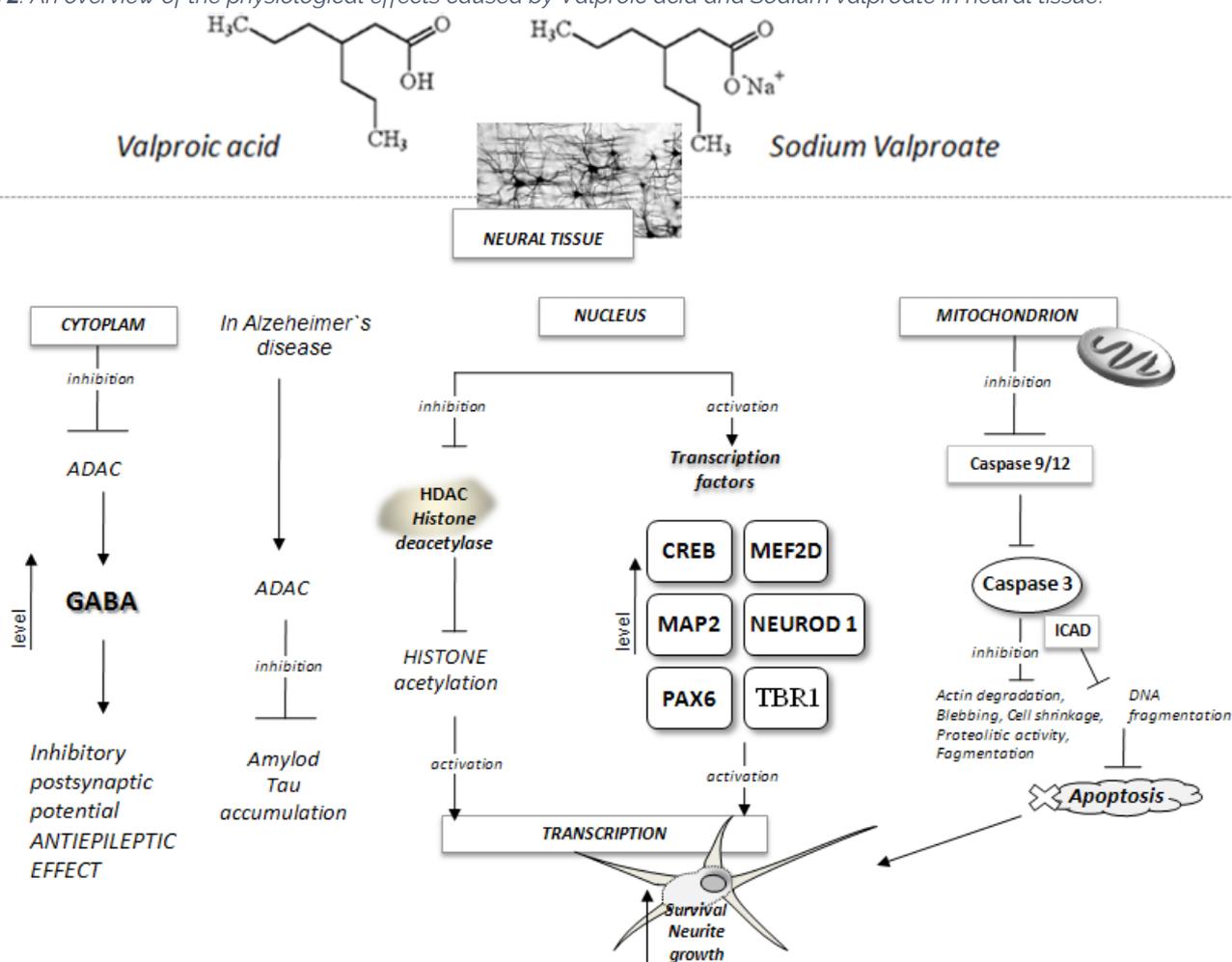
Figure 1. An overview of the physiological and adverse drug reaction effects caused by Valproic acid and Sodium Valproate treatment.



known metabolites. The VPA molecule is partially metabolized in the cytoplasm, and partially in the mitochondrion where it is transported by the help of carnitine. Within the cytoplasm, it is oxidized in Phase I reactions by cytochrome P-450(CYP450)-catalyzed oxidation with the major enzymes being CYP2A6, CYP2B6 and CYP2C9, which form major toxic metabolites 4-eneVPA and 2-ene VPA. Cytoplasmic VPA can be immediately conjugated to UDP-glucuronic acid by UDP-glycotransferases (UGT1A3, -1A4, -1A6, -1A7, -1A8, -1A9, -1A10 and -1A15). The 4-ene VPA and VPA molecules enter the mitochondrion conjugated to CoA (4-eneVPA-CoA and VPA-CoA) where they undergo mitochondrion-mediated β -oxidation and cytochrome P-450(CYP450)-catalyzed oxidation. The major metabolites of CYP450-catalyzed oxidation are the 2-propyl-4-pentenoic acid (4-ene-VPA), and the β -oxidation metabolite 2-propyl-2, 4-pentadienoic acid (2, 4-

diene-VPA). These metabolites are considered to be the main hepatotoxic metabolites of VPA. The metabolite 2-propyl-2-pentenoic acid (2-ene-VPA), which forms through the mitochondrial β -oxidation, is also converted to the 2, 4-diene-VPA *in vivo* (1;7;9). In Phase II of the biotransformation reactions, the majority of the formed metabolites are subjected to further glucuronidation, which is considered the major route for VPA and metabolite elimination. To a lesser extent, the adverse metabolite (2,4-diene-VPA-S-CoA) can be conjugated with glutathione (GSH) to form thiol conjugates, but more importantly it causes a decrease in mitochondrial glutathione levels, as described in rat hepatocyte cell cultures (9). The VPA glucuronides and their glucuronidated metabolites are excreted primarily by the urinary route and only in a small amount through the bile and into the intestines (1;7; 10).

Figure 2. An overview of the physiological effects caused by Valproic acid and Sodium valproate in neural tissue.



Mechanism of therapeutic action

It is thought that the major physiologic mechanism of VPA's therapeutic properties in epilepsy is the inhibition of 4-aminobutyrate aminotransferase (ABAT), a transaminase in the gamma aminobutyric acid (GABA) pathway. Therefore, VPA increases GABA release. It may also act by altering the properties of voltage-dependent sodium channels. In this way, it stops a seizure attack during epilepsy. VPA is also a histone deacetylase inhibitor. With this function, it promotes more transcriptionally active chromatin structures, which is a likely epigenetic mechanism for the regulation of many of the neuroprotective effects attributed to valproic acid. Intermediate molecules mediating these effects include the Vascular endothelial growth factor (VEGF), the Brain-derived neurotrophic

factor (BDNF) and the Glial cell line-derived neurotrophic factor (GDNF) (1, 10, 11).

Adverse drug reactions

Liver adverse effects

In the liver VPA causes an accumulation of lipids, cholesterol, and fatty acids with the subsequent development of hepatic steatosis and nonalcoholic fatty liver disease (NAFLD) in patients as well as in *in vitro* and *in vivo* models. The hepatic adverse drug reactions caused by steatosis are probably due to the abnormalities in lipid transport, but the exact mechanisms of liver adverse drug reactions are not clear. Neither are the mechanisms by which VPA induces nonalcoholic fatty liver disease (NAFLD). Experimentally on human HepG2 cell lines, *in vivo*, VPA treatment causes leakages of

ALT, AST, and LDH in a dose dependent manner suggesting injury to hepatocytes (12).

The 4-ene-VPA and 2, 4-diene-VPA metabolites and enzymes CYP1A1, CYP2A6, CYP2C9, ABCG1, and CPT1A are associated with hepatic adverse drug reactions in humans and in certain hepatic cell lines (8;12). However, only the CYP2A6 polymorphism was found to be associated with higher concentrations of 4-ene-VPA and 2, 4-diene-VPA. Potential important risk factors include mutated genotypes of CYP2C9 and CYP2A6, and higher concentrations of VPA (8).

Exposure to sodium VPA has been shown to induce a down regulation of several transcripts in cultured hepatocytes. Their low levels resulted in time dependent fluctuations of cellular ATP, which can lead to cell death (13).

Hepatic adverse drug reactions caused by VPA have also been associated with mitochondrial dysfunctions, with the inhibition of enzymes in the beta-oxidation pathway and with oxidative stress. As aforementioned, VPA can form thiol conjugates particularly with glutathione (9). Such decreases of intracellular glutathione levels are known to change the redox status of the cell and lead to the adverse drug reactions of oxidative stress effects. For example, VPA incubation of rat hepatocytes in vitro caused glutathione depletion which suggests a possible cellular oxidative stress after VPA exposure (9). For VPA, the role as a potential oxidant is underexplored, and there are scarce literature sources on the subject. Experiments on human HepG2 cell lines showed that exposure to VPA for more than 72h increased levels of mitochondrial reactive oxygen species production (ROS), but decreased protein levels of mitochondrial superoxide dismutase SOD2, suggesting oxidative stress caused by impaired elimination of mitochondrial ROS (13).

Pourahmad et al. (14) and Jafarian et al. (15), both, in vitro, detected increases in ROS formation along with a decrease in mitochondrial membrane potential upon the treatment of rat liver mitochondria with VPA; all of these were events before cell death signaling began. Specifically, Jafarian et al. (15) showed that ROS

is associated with increased lipid peroxidation, mitochondrial membrane collapse; mitochondrial swelling and finally the release of cytochrome c. release began apoptotic cell death signaling. Similarly, Pourahmad et al. (14) in their experiment demonstrated that the cytotoxic action of VPA manifests itself as lysosomal membrane leakiness in conjunction with ROS formation and a decline in membrane potential. Again, all were events before cell lysis started.

Chang et al. (16) investigated the genetic polymorphisms in the G protein beta three subunit (GNB3) and associations with the metabolic phenotypes of VPA treated human patients. Their study confirmed that patients, who are T allele carriers of GNB3 C825T polymorphism, have a lower risk of VPA induced metabolic abnormalities. They warrant further studies investigating mechanisms of VPA induced metabolic abnormalities and G protein.

An article by Stewart et al. (17) describes that the risk of hepatic adverse drug reactions induced by VPA is increased in patients with mitochondrial diseases, especially those with POLG1 gene mutations. POLG genes code for the mitochondrial DNA polymerase gamma and their mutations can cause Alpers-Huttenlocher syndrome, a neurometabolic disorder linked to an increased risk of developing fatal hepatic adverse drug reactions upon exposure to VPA. Stewart et al. (17) also described an association of VPA induced hepatic adverse drug reactions with a genetic variation in the POLG gene. In their case, it was a heterozygous genetic variation in the POLG gene which was primarily due to the p.Q1236H substitution, and it was strongly associated with VPA induced liver toxicity. In their experiment on primary human cell lines, therapeutic doses of VPA stopped human cellular proliferation and high doses of VPA induced non-apoptotic cell death, not mediated by mitochondrial DNA depletion, a defect in fatty acid metabolism or mutation. Therefore, another mechanism of liver injury by VPA is impaired cellular liver regeneration. One of the ways of preventing it is by prospective genetic testing for POLG which could identify individuals at high

risk for potentially fatal consequences of VPA treatment (17).

Hepatic lipid metabolism is impaired during VPA treatment. The secretion of triacylglycerols and phospholipids at the sinusoidal pole of hepatocytes are reduced by an acute administration of VPA. This inhibition of secretion has been thought by Berllinger et al. (18) to be a factor in the development of steatogenic hepatic adverse drug reactions by VPA treatment (18) in rat hepatocytes, though so far shown only in an animal model.

Gene expression profiles in hepatocytes could be associated with the steatogenic hepatic adverse drug reactions of sodium VPA. Gene profiling data showed striking changes in the expression of genes associated with lipid, fatty acid, steroid metabolism, oncogenesis, and signal transduction and development in mice treated with VPA (19). Lee, et al. (19), found that 1156 genes were up and down regulated after exposure to sodium VPA. 60 genes were involved in lipid metabolism and were interconnected with the biological pathways for biosynthesis of triglycerides and cholesterol, catabolism of fatty acids and lipid transport (19).

Obesity, fatty acid, and cholesterol disorders in patients treated with VPA

Chronic treatment with VPA is commonly associated with weight gain, which potentially has important health implications, in particular increased central fat distribution. A positive correlation between chronic treatment with VPA and increased abdominal weight as well as increased blood pressure was recorded previously in human patients (20). An association was found between VPA induced weight gain and insulin resistance, hyperinsulinemia, hyperleptinemia and leptin resistance. Furthermore, the patients who had VPA induced weight gain were more likely to develop metabolic syndrome and dyslipidemia with associated long term vascular complications such as hypertension and atherosclerosis. In addition, they stated that long term VPA therapy carries a risk for atherosclerosis because of an accumulation of oxidative stress in combination

with elevations of uric acid and homocysteine. Another experiment in human patients showed that VPA induced weight gain in the experimental group taking Valproate but noted weight gain was not due to decreased physical activity. In these participants of the study, there was a noted increased motivation to eat and decreased glucose levels in the experimental group compared to control group (21). Interestingly, quite opposite to these results in humans, Khan et al. (22) in their recent work in animal models discuss that the reason for the weight increase with VPA therapy might be increased appetite and irregular thirst, and consequent over-consumption of energy-rich alimentary. Also, they point out the dependence on the doses and duration of treatment, which changes the pharmacological signaling, differences in clinical and preclinical findings, as well as inter-species variability. The authors highlighted the role of histone deacetylases (HDACs) in insulin-resistance, gluconeogenesis and islet function, and formed a hypothesis that VPA treatment (in particular dose) might have a beneficial role in diabetic disorder. HDACs can modulate the expression of various genes, which directly or indirectly affect glucose metabolism. In their experimental work, Khan et al. (22), proposed and recorded the anti-diabetic role of VPA by the modulation of insulin signaling and forkhead box protein O1 (FOXO1)-mediated gluconeogenesis in type-2 diabetic Sprague-Dawley rats. In the proposed model diabetes was induced by the combination of a high-fat diet and low dose streptozotocin, and VPA was given at the doses of 150 and 300 mg/kg/day. VPA treatment significantly reduced the plasma glucose, HbA1c, insulin-resistance, and the fat deposition in the brown adipose tissue, white adipose tissue and liver, which is comparable to metformin effects—which the authors used as a positive control to create equal effects. The treatment also inhibited the gluconeogenesis and glucagon expression and recuperated the histopathological changes in the pancreas and liver. Detailed molecular mechanisms of the findings might elucidate the possible physiological mechanisms.

Adipokines

As expressed in the previous chapter, in recent years, research focused on adipokine signaling to elucidate the VPA effects in increased obesity. It seems that without doubt, VPA affects the neurohormonal regulation of appetite, as confirmed in both patients and animal models. Weight gain during VPA treatment may be related to increases in leptin, insulin and neuropeptide Y (NPY) levels. Tokgoz et al.(23), conducted a study on 20 prepubertal children which compared the BMI, leptin, insulin, NPY, adiponectin and ghrelin levels with lipid profiles, and CAIMT(carotid artery intima media thickness), before and after a treatment period with VPA of six to twelve months. The study aimed to evaluate and to determine whether these parameters indicate the development of early atherosclerosis. The treatment did not affect plasma ghrelin, adiponectin levels, lipid profiles, and CAIMT after 6 and 12 months of treatment compared with pretreatment values. Although BMI and the appetite regulatory molecules were changed, early atherosclerotic changes were not triggered within a year of treatment (23). A similar study on VPA therapy, conducted earlier on 18 children (10.94 ± 3.78 years), showed, after 18 months of VPA exposure, an increase in the serum leptin, insulin, and neuropeptide Y (NPY) levels, but also in the glucose, cortisol, galanin and ghrelin levels compared to the matching control. There was an increase of 2.3kg of body weight combined with an increase in named obesity signaling molecules in the treatment group compared to the control group (24).The findings of these two studies of decreased ghrelin levels and increased weight gain are in stark contrast to the findings of the Gungor et al. (25) group which found increased levels of ghrelin in proportion to an increase in weight of prepubertal patients. Meral et al. (26) included 44 children with idiopathic, generalized epilepsy treated with valproic acid (VPA) as an experimental group, and 40 children without therapy as the control group in their study (26). Neither the VPA treated group nor the control group showed any significant difference in terms of LDL cholesterol, total cholesterol and age. However,

the VPA treated group subjects had significantly higher BMI-SDS as well as higher levels of visfatin, apelin, and triglycerides, but lower levels of adiponectin and HDL levels than the control subjects. The group concluded that visfatin, adiponectin and apelin can be considered as potential regulators of fat and glucose metabolism during valproic acid therapy (26).Grosso et al.(27) found that patients with VPA associated obesity had high concentrations of leptin in their blood. They did not find any differences in leptin concentration between patients who had VPA induced obesity and obese controls during their experiment. Li et al. (28) found that VPA can increase serum lipid levels in both juvenile and adult rats, but that higher levels of lipids could be found in juvenile rats (28).

Ghrelin is affected by valproic acid treatment and there are potential effects of such interactions on weight gain.A study on 35 pediatric patients aged three to fifteen years were evaluated for ghrelin, leptin, C-peptide, insulin, insulin like growth factor- 1, insulin like growth factor binding protein- 3 and glucose levels.Serum ghrelin levels were increased significantly with a negative correlation with insulin like growth factor-1 and insulin like growth binding protein 3 in the prepubertal group at six months of treatment. Thus, the weight gain caused by valproic acid could be linked to the increased levels of ghrelin levels in the early treatment period (25).

Polycystic ovarian syndrome (PCOS)

A higher occurrence of polycystic ovarian syndrome (PCOS) compared with other antiepileptic drugs is one of the major adverse drug reactions reported in women treated with VPA (29;30). Several molecular mechanisms could account for this epidemiological appearance. VPA treatment can be connected to hyperandrogenism, hyperandrogenemia, oligoovulation, the appearance of PCO on an ultra-sonogram, elevated levels of testosterone, and irregular menstrual cycles. The incidence of occurrence of PCOS in women taking VPA is 1.95 times higher than in women

being treated with other antiepileptics (31). It seems that VPA treatment during pregnancies possesses a significant risk of teratogenic effects based on a survey where a 15 out of 229 (6.6%) women prescribed valproate gave birth to a child with a major congenital malformation (29).

Since testosterone levels slightly increase, and progesterone and estradiol levels decrease over a longer treatment period with VPA, it is believed that such an imbalance induces polycystic ovarian changes and menstrual suspensions (amenorea) that later lead to polycystic ovarian syndrome (32).

Indeed, some authors, who also recorded increased levels of testosterone in women treated with VPA for bipolar disorder, proposed that a broad inhibitory action on glucuronidation systems and on cytochrome systems causes high concentrations of testosterone, dehydroepiandrosterone sulfate, and androstenedione. VPA treatment over a longer period of time is associated with increased levels of testosterone. It is also associated with a development of menstrual abnormalities. There is a significant correlation between VPA treatment and a reduction in the levels of mRNA encoding estrogen receptor alpha (ER alpha), which causes a lack of ER alpha protein in breast and ovarian cell lines. Beside the regulation of sex physiology and menstrual abnormalities, the weight gain and osteoporosis could be a result of the lack of estrogen signaling because of the clearance of ER alpha protein in cell lines (33). Furthermore, beside the VPA interference with the peripheral endocrine hormones, at the central regulatory level of the hypothalamus-hypophysis axis, VPA also causes decreased excretion of LH (luteinizing hormone), FSH (follicle stimulating hormone) and prolactin. Together, the steroid sex hormone imbalance, the hyperinsulinemia and obesity in humans, which occur due to VPA treatment, further contribute to an increase in sexual dysfunctions (34).

Impairment of synthesis of steroid hormones

Mechanisms of ovarian toxicity include a possible disruption of the pathways for the synthesis of sex hormones as a major cause in the development of PCOS. Brion et al. (35) expressed that VPA may increase mitochondrial cholesterol transport by a mechanism independent of the steroidogenic acute regulatory protein, as proven in an experimental model.

Some authors explain the hormonal imbalance as a direct consequence of differential activity on the expression of CYP enzymes involved in hormone synthesis, which impairs the conversion of testosterone to estradiol (36, 37). A suppression of aromatase expression takes place in granulosa cells as an answer to treatment with VPA. For example, the follicular development of 14-day-old rats was suppressed, and testosterone, estradiol, androstenedione, and the combined levels of all steroid hormones tended to decrease over time with exposure to VPA (38). Gustavsen et al. (37), found *in vitro*, that the expression of genes coding for enzymes early in steroidogenesis was downregulated. Such changes did not occur with the use of other antiepileptics (37).

The suppression of aromatase expression means that the synthetic pathway from cholesterol to estradiol, including the *de novo* synthesis of cholesterol, is suppressed (38). Contrary to these *in vivo* results in isolated mitochondria, *in vitro*, VPA stimulates exogenous cholesterol conversion to progesterone (38). This indicates that the complex physiological mechanisms do not fulfill the whole picture of physiological interactions as *in vivo*.

The majority of the mechanistic studies on steroidogenesis of sex hormones and ovarian hyperandrogenism rely on *in vivo* and *in vitro* investigation of VPA exposure only, and measurements of CYP conversion in patients is scarce. There are inconsistencies in the literature regarding the effects of VPA on the female reproductive steroidogenesis, as pointed out by Glistler et al. (39) in their review of literature on the subject. *In vivo* in rodent models, Glistler et al. (39) mention both the increase in the number of

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follicular "cysts" and total ovarian weight with decreased plasma testosterone level, then list experiments with no effect on serum androgen levels. However, reduced serum estrogen levels affect the androgen/estrogen ratio, where androgen hormones become relatively higher. Interestingly, Glister (39) mentions the experiment on primates where VPA treatment in a Rhesus monkey with normal cycling had no effect on androgen levels or ovarian morphology. The authors (39) further list similar inconsistencies in the in vitro studies with some experiments: (i) recordings of increased ovarian androgen synthesis and an increased transcription of steroidogenic genes, (ii) an inhibitory effect of VPA on hCG-induced androgen secretion, (iii) increased basal and LH-stimulated androgen secretion or decreased LH-stimulated androgen secretion and reduced basal and FSH-induced estradiol secretion. In their own experiment (39) in primary bovine theca (TC) and granulosa (GC) cells (a model closely relating human cycling), they exposed TC to VPA (7.8–500 µg/ml) with/without LH and GC with/without FSH or IGF analogue. In Theca cells, VPA reduced basal androstenedione secretion by 70% and in VPA/LH-induced theca cells by 93%. CYP17A1 mRNA was reduced by more than 99% and LHR, STAR, CYP11A1 and HSD3B1 mRNA was also lower. VPA only reduced theca cells progesterone secretion induced by the highest (luteinizing) LH dose. At higher concentrations (125–500 µg/ml) VPA inhibited basal, FSH- and IGF-stimulated estradiol secretion in granulosa cells without affecting progesterone secretion. VPA reversed FSH-induced upregulation of CYP19A1 and HSD17B1 mRNA levels. Vice versa, VPA inhibits both LH-dependent androgen production and FSH/IGF-dependent estradiol production. The authors proposed that the named changes are consequences of the HDAC inhibitory properties and conclude that the VPA has a direct stimulatory action on theca cell androgen production (39).

Alzheimer's disease and VPA, new perspectives

In recent work, evidence was gathered that besides GABA promoting effects, VPA treatment has antiapoptotic and protective effects in neural tissue (Figure2), though as with steroidogenesis, mostly in model experiments. The deinhibition of histone acetylation caused by histone deacetylases (HDACs) inhibitors could contribute to the recovery of learning and memory in rats. Histone hypoacetylation of lysine residues contributes to cognitive impairments in Alzheimer's disease (AD). VPA can significantly elevate histone acetylation through HDAC activity inhibition. Experiments showed that VPA treatment can boost the long-term recognition memory and spatial learning memory in AD transgenic mice. As such VPA could significantly improve cognitive function in AD (40, 41).

Experimentally, VPA ameliorates spatial memory impairment and amyloid beta deposition in transgenic mice (42). VPA treatment caused a decrease in senile plaque formation, and Amyloid beta 40 and Amyloid beta 42 accumulations. Several studies, including those by Longet al. (43) in 2016, proposed that gender differences play a role in determining how well VPA affects the treatment of Alzheimer's disease. It appears that gender differences play a role in the VPA effects on AD, since these effects were more notable in the male than in the female AD mice (43).

As a histone deacetylase inhibitor, VPA is able to upregulate neprilysin (NEP) expression and activity in human neuroblastoma SH-SY5Y cell lines, which usually express very little NEP protein. Upregulation of expression and activity of NEP in the rat hippocampus was, also, observed following i.p. injections of VPA to rats. NEP is an amyloid degrading enzyme which in the healthy brain maintains Amyloid beta levels at physiologically low concentrations. The activity and expression of these enzymes decreases with age and, with some other pathological conditions, predisposes to late onset of AD (40).

The synapse damage caused by amyloid beta protein was reduced by pretreatment with physiologically relevant concentrations of VPA

(10 μ M). VPA also decreased synaptic damage caused by other neurodegenerative associated proteins such as alpha synuclein, which is linked to Lewy Body dementia and Parkinson's disease (44).

VPA treatment inhibited the activity of GSK-3 β , decreasing hyper phosphorylated Tau by lower phosphorylation, as seen in the transgenic mice (45).

One of the main reasons of neuronal loss in AD is apoptosis of neurons. It seems that in neural tissue, VPA promotes the reduction of apoptosis by blocking and decreasing apoptotic signals and increasing survival (46). Evidence for reduction of apoptotic signals includes a significant reduction in the expression of interleukin 1-beta (IL-1 β) and the tumor necrosis factor alpha (TNF- α), as well as the micro and astrogliosis in the hippocampus and cortex of APP/PS1 transgenic mice (43). Besides the reduction of antiapoptotic signals, these results imply that VPA has anti-inflammatory properties in the brain that are opposite of the effects it has in the liver.

Further evidence of a decrease of apoptotic signaling in the mouse AD model showed that VPA acted via the suppression of both the mitochondrial and the endoplasmic reticulum pathways of apoptosis, by downregulating the expression of Caspase 3, Caspase 9, Caspase 12, and by reducing the level of cytochrome C and Bax. Antiapoptotic protein BCL 2 expression was increased, intracellular levels of Ca²⁺ decreased, and the mitochondrial membrane potential was elevated (46). These are yet other effects of VPA which seem to be completely opposite of those that are occurring in the liver where apoptosis inducing effects were demonstrated, as described previously in the text.

Most interestingly, beside the antiapoptotic effects, the proliferative effects in neurons, increase in synaptogenesis and novel connectivity between neurons are also important novel physiological roles of VPA in neural tissue. Experimentally, VPA caused an increase of CREB and BDNF expression, causing accelerated neurite outgrowth, modification of synaptic structure and improvement of

behavioral deficits in AD mouse models. Accumulating evidence supporting such research included reports where VPA was able to induce MAP 2 gene expression, which mediated the process of de novo re-arborisation and neurite outgrowth of neurons. These functions add to the process of successful neuronal re-wirings (47). VPA treatment includes the activation of regulatory pathways that enhance neurogenesis and suppress gliogenesis. Genes which encode the transcription factors (TFs) that specify neuronal cell fate, including MEF2D, MYT1L, NEUROD1, PAX6 and TBR1, and their target genes, are induced by VPA (48). Neural stem cells (NSCs) derived from Niemann-Pick Type C disease (NPC) mice, a neurodegenerative and lipid storage disorder for which no effective treatment is known, showed impaired self-renewal and differentiation. VPA was able to induce neuronal differentiation and restore impaired astrocytes in NSCs from NPC1 (-/-) mice. Increasing levels of cholesterol within NSC from NPC1 (-/-) mice could be reduced by VPA. Necessary neurotrophic genes (TrkB, BDNF, MnSOD, and NeruoD) were upregulated through the repression of the REST/NRSF and HDAC complex by VPA treatment. These upregulated neurotrophic genes were able to enhance neural differentiation and cholesterol homeostasis in neural stem cells from NPC1 (-/-) mice (49). Such protective and beneficial role in AD, tau and amyloid accumulation will be the future of VPA research.

Discussion and conclusion

VPA is a short, branched chain fatty acid with strong anticonvulsant properties. It has diverse effects in different tissues, with opposing mechanisms of action that seem to be dose and exposure-time dependent (Figure 1). The number of papers published so far (Table 1), including approximately several hundred papers each year in the last decade, in all areas of physiological action, on both models and patients (Table 2), prove that the research on VPA physiology still raises interest in basic physiological research. The literature cited in this work, divided by subject area of research and

Table 2. Cited literature on Valproic acid and/or Valproate, divided into categories by subjects of proposed physiological mechanisms and scientific research methods *in vitro*, *in vivo* and epidemiological studies on patients..

Physiological Parameter	In vitro (Cell culture)	In vivo (Animal models)	Human patients (case, clinical and epidemiological studies)	No. of cited papers (TOTAL)
Doses and therapy			1;2;3;4;5;6;10	7
Biotransformation	7;9;11;14	38	7;8;11;16	9
Liver adverse effects	9;12;13;14;15;17;18	18;19	8;17	11
Gonads, hormones, sexual physiology adverse effects	29;33;35;36;37;38	38	30;31;32;34	11
Steroidogenesis adverse effects (biotransformation enzymes in sex hormones biochemistry)	35;36;37;38;39			5
Overweight, BMI, adiposity			16;20;23;24;25;27	6
Triglycerides, fatty acids cholesterol (biochemical mechanisms, adverse effects)	18;49	18;19;28	16;26	7
Adipokines		28	16;20;21;23;24;25;26	8
Glucose and insulin, energy metabolism	22	29	16;21;23;24;25	7
Oxidative stress	13;14;15			3
Neural tissue beneficial effects, synaptogenesis, neural growth and beneficial effects in Alzheimer disease models	44;49	40;41;42;43;44;45;46;47;48		11
No. of cited papers (TOTAL)	30	17	37	

type of research (models and patients) (Table 2), is mainly from the last few years of research with only a few articles extended to the previous decade. From the comparison of cited physiological mechanisms, several major questions arise that will shape further research.

Although a relationship between VPA therapeutic mechanisms and GABA action is generally accepted, the mechanisms of its

adverse reactions in other tissues have not been fully elucidated. The major questions that will impact future research directions are whether there is a unique fundamental physiological mechanism that triggers different reactions in the tissues with opposing action, or whether the different tissue responses are a consequence of diverse target pathways induced by VPA. Answering this fundamental question will direct

and focus the research toward a mechanistic explanation of adverse effects and hypothetical novel properties, such as a model based beneficial mechanism in Alzheimer's disease. The beneficial effects in neural tissue is mainly gained hypothetically in the models (Table 2) and has yet to be proven in human subjects. The models based on a novel proposed beneficial mechanism in neurons and especially in the protective role in the forming of Alzheimer's disease should also investigate the potential of VPA to prevent the transmigration of accumulated Tau and Amyloid in already formed plaques within the neural tissue. Research of such therapeutic potential in patients with early signs of plaque formation would be more important than VPA preventive potential, since it is highly unlikely that the healthy patients could be subjected to therapeutic exposure to VPA as a measure of prevention, even if the beneficial effects in prevention of the first steps of accumulation, and hyperphosphorylation of these molecules are proven in humans.

The problem of weight gain and energy physiology caused by the treatment with VPA still remains unsolved and limits all beneficiary actions described in neural tissues. Although noted in patients and animal models, the adverse drug effects on adiposity and lipid accumulation has not been clearly explained. It is believed that the hepatocyte mitochondrion disturbance is a major cause of imbalance of lipid metabolism in the liver and one of the causes of weight gain observed in patients treated with VPA. However, even though some genetic predispositions (such as POLG mutation) have been detected, it seems from the cited references that the effects could be more epigenetic in nature, and future research should focus on designing experiments connecting targeted genetic predisposition (mutations) and epigenetics. Furthermore, the hepatocyte lipid imbalance should be further investigated physiologically from the point of major conversing enzymes along the biochemical pathways of triglyceride, fatty acid and cholesterol synthesis/biochemical redistribution and lipoprotein synthesis and

redistribution (for example Acetyl-CoA carboxylases 1 and 2 (ACC1 and ACC2), fatty acid synthetase (FAS), Stearoyl-CoA desaturase-1 or carnitine/palmitoyl-transferase 1 etc.). References in this direction of research are missing. Besides the proposed hepatic adverse drug reactions that influence lipid metabolism and transport, recently, the research concentrated on the role of appetite regulation pathways involving adipokines and other metabolic regulating hormones. This subject is abundant in the literature with both evidence from the patients and animal models. The exact question that future experimental design has to focus on would be whether adipokine disturbance (increase) is directly unleashed by VPA action as a cause or whether it is a consequence of adipocyte (and GI) reaction to differential fatty acids. There is a probability that adipokine activation and appetite enhancements are merely a consequence of disturbed lipid physiology within the adipocytes or hepatocytes. Besides lipid physiology, due to the abnormal effects on energy, fatty acid, and cholesterol metabolism, VPA treatment is a risk factor for the development of other metabolic diseases with its associated complications such as atherosclerosis, and metabolic syndrome, while in the CNS it has many beneficial effects. Research should also focus on the physiological pathways of cholesterol transport and metabolism not only in experimental models (Table 2), but in the liver, reproductive organs (steroidogenesis) and in neural cells of patients as well.

It seems that in the last decade the research field of oxidative stress has been abandoned or reduced in vivo (Table 2). In addition to the biochemical pathways of lipid metabolism, the cellular redox status of biochemical balance might also be important in contributing to the differential activation of physiological pathways of lipid metabolism and epigenetic changes that may enhance pathophysiological changes. Thus, future studies on VPA physiology should neither neglect this area of research. Within all prospect studies, special attention should be given to the doses of exposure.

In conclusion, although prescribed as an effective anticonvulsant, most of the pathogenic pathways of VPA's unwanted and beneficial molecular effects are not fully understood and further studies and experiments are warranted.

Disclosures

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