Biochemical, Molecular and Epigenetic Mechanisms of Valproic Acid Neuroprotection

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Abstract: Valproic acid (VPA, 2-propylpentanoic acid) has been widely used as an antiepileptic drug and for the therapy of bipolar disorders for several years. Its mechanism of action was initially found to be primarily related to neurotransmission and modulation of intracellular pathways. More recently, it emerged as an anti-neoplastic agent as well, by acting on cell growth, differentiation and apoptosis. Here, it mainly exerts its effect by regulating gene expression at the molecular level, through epigenetic mechanisms. In particular, it has been demonstrated the effect of VPA in chromatin remodeling, as VPA directly inhibits histone deacetylases (HDACs) activity. Interestingly, it has been observed that these biochemical and molecular pathways are involved not only in beneficial effect of VPA against epilepsy and malignancies, but they are also responsible for more general neuroprotective mechanisms. In particular, it has been demonstrated that VPA is neuroprotective in several models of neurodegenerative diseases. Moreover, due to the involvement of the VPA-affected mechanisms in complex behaviors, VPA is increasingly used as a psychotherapeutic agent. This review summarizes the more recent data on VPA neuroprotective mechanisms at the biochemical, molecular and epigenetic levels, focusing on both *in vitro* and *in vivo* models of neurodegenerative diseases. In particular, attention is paid to mechanisms by which VPA affects neuronal survival/apoptosis and proliferation/differentiation balance, as well as synaptic plasticity, by acting both directly on neurons and indirectly through glial cells. Perspective applications of the VPA neuroprotective potential in human neurodegenerative diseases are discussed, when relevant.

Keywords: Valproic acid, neurodegenerative diseases, neuroprotection, signal transduction, transcription factors, histone acetylation, epigenetic mechanisms.

INTRODUCTION

Valproic acid (VPA, 2-propylpentanoic acid), a short branched chain fatty acid, was originally synthesized as an analog of valeric acid extracted from *Valeriana officinalis* and it has been used worldwide for decades, in the form of sodium valproate, as an antiepileptic drug with therapeutic value for absence, partial and tonic-clonic seizures [1]. The success of VPA-based therapies has made branded products containing it and sold by major pharmaceutical companies top scorers among antiepileptic drugs [2, 3]. However, the need for high dosages of the drug and the occasional occurrence of serious side effects, teratogenesis and liver toxicity, has prompted the search for second generation VPA drugs provided with better efficacy and lower side effects [2]. While VPA, similar to other antiepileptic drugs, shows clear efficacy in suppressing epileptic seizures, it is less clear from epidemiological studies and meta analysis of clinical trials whether these drugs also exert an anti-epileptogenic curative effect [4-7]. The answer to this question is obviously complicated by the fact that, in a significant number of cases, some forms of epilepsy undergo spontaneous remission, making it necessary to evaluate any long-lasting curative effect in terms of increased chance of remission [8]. The pharmacological basis of the antiepileptic action of VPA has been related to multiple effects contributing to reduction of neuronal excitability [9]. These include increase of GABAergic activity [10-14], negative regulation of NMDA receptor-mediated glutamatergic excitatory activity [15-18] and limitation of frequency of action potentials through negative regulation of voltage gated Na^+ channels [19, 20].

The recent discovery [21, 22] that VPA is an effective inhibitor of histone deacetylases (HDACs), the key enzymes for the control of histone acetylation state and hence for the epigenetic regulation of gene expression, has opened novel perspectives towards the molecular understanding of the drug action, as considered in detail in several places of the present review. Initial anecdotal observations of VPA efficacy as an anti-manic drug and as an effective agent in the treatment of migraine were subsequently substantiated by clinical trials [23]. In parallel, initial observations of anti-proliferative effect of VPA have prompted several studies aimed at establishing its role as a chemotherapeutic in cancer treatment [23]. These actions of VPA will be briefly considered in the next paragraphs of this section.

VPA has been used for treatment of some mood disorders starting from mid-70s [24, 25]. This is, in particular, the case of bipolar depressive syndromes, for which the drug is particularly effective towards manic symptoms, while it only shows marginal antidepressive effects [26-32]. Early studies in this field brought to approval of VPA as an anti-manic drug from FDA in 1995. Psychomotor agitation and severe depression agitation are associated with major depression syndromes and may be a risk factor for suicidal behavior [33]. Treatments with VPA-based drugs have demonstrated efficacy towards depressive agitation [34]. These drugs are also effective to treat agitation and aggressive behavior in patients with personality disorders or mental retardation [35-37], as well as in patients in which agitation is associated with dementia [38, 39]. In addition to ameliorate behavioral disturbances, VPA has been recently reported to be potentially beneficial for cognitive problems in elderly dementia [40]. As VPA, similarly to lithium, possesses among several other biochemical cellular effects the ability to decrease the efficiency of inositol-related signaling into the cell, a "inositol-depletion hypothesis" was formulated to explain the efficacy of lithium, VPA and related drugs on bipolar mood disorders. While this hypothesis has been worked out for a couple of decades, conclusive evidence of its validity has been not yet provided, as discussed in a recent review[41].

Anecdotal observations reporting migraine amelioration in patients using VPA for epilepsy treatment brought to more controlled studies on anti-migraine effects of VPA administration [23]. These initial observations evolved in controlled clinical trials that established this therapeutic role [42-45]. The use of VPA for migraine was approved by FDA in 1996 and VPA-based drugs have been since then widely used to this therapeutic purpose.

Anti-tumor properties of VPA emerged with the demonstration that the drug was able to inhibit growth of several types of cancer cells and to promote their differentiation [46-48]. The differentiative action of VPA towards tumor cells has been often described to be accompanied by induction of apoptosis, either related to caspase activation or not [49-51]. A further anti-tumor action of VPA is related to its ability to inhibit angiogenesis in the cancer tissue [52, 53]. An interesting property of VPA, a drug whose prolonged use at high concentrations is relatively safe, is that it is able to enhance the effect of other anti-tumor treatments. This is true concerning, for

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instance, association of VPA with retinoic acid or with interferon alpha [54-58]. Recent evidence extends the cooperative role of VPA in tumor treatment to radiation therapy as the drug enhances the sensitivity of human cancer cells to ionizing radiations [58]. Clinical trials based on VPA mono-therapy or on combination therapies are ongoing [47, 48].

The extent of the pharmacological actions of VPA briefly summarized in this chapter, in addition to those related to neuroprotection that will be considered later in this review, suggests that the drug is likely affecting several fundamental cellular processes, by targeting multiple molecular mechanisms. In the following sections, the present status of our knowledge regarding these mechanisms will be reviewed, focusing in particular on those related to the improvement of essential neural function and to neuroprotection. As several mechanisms and effects of VPA are shared by lithium, similarities or differences in the action of the two drugs will be considered when relevant.

BIOCHEMICAL AND MOLECULAR EFFECTS OF VALPROIC ACID

Regulation of the glutamate excitatory neurotransmission and/or gamma aminobutyric acid (GABA) inhibitory neurotransmission are among the better characterized mechanisms of VPA in mood regulation. Similar to lithium, intracellular mechanisms of VPA also include regulation of several protein kinase signaling pathways and of gene expression. All of these mechanisms of VPA action will be discussed in the following sections.

Effects on the GABAergic and Glutamatergic Systems

The effect of VPA on the GABAergic system has been postulated from the 70's [59, 60]. The drug enhances inhibitory GABAergic activity either by increasing availability of synaptic GABA and/or by enhancing postsynaptic GABA responses. VPA increases GABA levels and, therefore, GABA-mediated postsynaptic inhibition in cultured mammalian neurons [61]. Moreover, VPA treatment elevates GABA levels in human plasma [62] and in several rat brain regions [63]. While contrasting results are reported regarding VPA effects on GABA uptake and release [64-68], an unanimous consensus exists on the fact that the main mechanisms through which VPA increases GABA levels is through its metabolic pathways. As shown in Fig. (**1**), VPA inhibits GABA catabolism [69], by decreasing its degradation mediated by GABA transaminase [70, 71] and succinate semialdehyde dehydrogenase (SSA-DH) [72]. In addition, VPA elevates GABA levels, both by increasing the availability of its precursor α-ketoglutarate, through the inactivation of α-ketoglutarate dehydrogenase [73], and by enhancing the activity of glutamate decarboxylase (GAD), the key enzyme for GABA synthesis [70, 74]. Recently, it has been observed that VPA affects not only the catalytic activity, but also the expression of GAD, due to its action on histone acetylation [75, 76]. Moreover, VPA affects GABAergic neurotransmission by acting on GABA receptors too, enhancing responses of both GABA-A and GABA-B receptors [77-79]. In particular, VPA prolongs the decay time of the post-synaptic inhibitory response, by interacting with the benzodiazepine regulatory site of the GABA-A receptors [79], and it increases the baclofen binding to GABA-B receptors [78].

Glutamatergic transmission is a major target for VPA [80] which decreases brain levels of glutamate and aspartate, while increases taurine content, an effect that could be related to anoxia tolerance [81]. In astrocytes, acute exposure to VPA decreases the transport of glutamate and aspartate while chronic exposure has no effect on their uptake kinetics [82]. In the same cells, VPA enhances the activity of glutamine synthetase, both in culture and *in vivo* [83, 84]. Similarly to lithium, VPA stimulates glutamate release from cortical slices, but the effects of the two drugs are additive, suggesting that they use different pathways [85]. At clinically relevant concentrations, VPA inhibits glutamate binding to AMPA receptors [86], an effect that may not be directly linked to neuroprotection [87], and it also regulates the synaptic expression of AMPA glutamate receptor subunit 1 (GluR1). Chronic (but not acute) VPA treatment reduces hippocampal synaptosomal GluR1 levels and decreases GluR1 phosphorylation at a specific PKA-sensitive site [88]. This VPA effect contributes to the modulation of the excitatory activity of glutamate in medial prefrontal cortex, which also involves NMDA and kainate receptors [18]. VPA significantly enhances NMDA receptor-mediated transmission and increases plasticity in the neocortex through a selective over-expression of NR2A and NR2B subunits of NMDA receptors, as well as of the calcium/calmodulin-dependent protein kinase II (CAMK-II) [89].

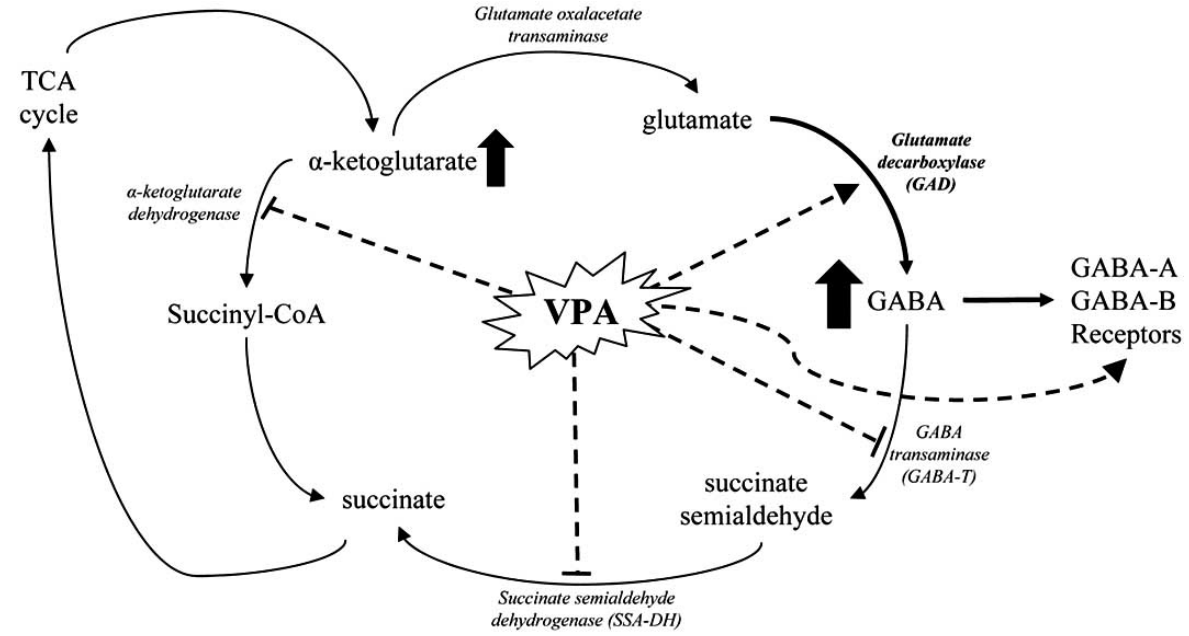

Fig. (1). Mechanisms of VPA action on the glutamate and GABA metabolism. The dashed lines indicate the enzymes affected by VPA (⊥ means inhibition; → indicates induction), while the bold arrows up indicate the VPA increasing effects on α-ketoglutarate and on GABA.

VPA also causes an increase of the glutamate-aspartate transporter (GLAST) protein measured in the hippocampus and a decrease in the glial glutamate transporter-1 (GLT-1), while the neuronal excitatory amino acid carrier-1 (EAAC-1) is unchanged [68]. The final result of these contrasting effects is regionally different, as chronic treatment of rats with VPA dose-dependently increases the hippocampal glutamate uptake capacity, while no effect of VPA on glutamate transport is seen in frontal or parietal cortices or in cerebellum [90]. Indeed, increased glutamate uptake seems to importantly contribute to VPA-mediated neuroprotection in striatum [91].

Taken together, the effects of VPA on the two major brain neurotransmitter systems only partially justify the antiepileptic and neuroprotective role of the drug. While VPA clearly potentiates GABAergic activity, thus playing a putative role in dampening dangerous excessive excitation, its role in glutamatergic transmission is less straightforward, as it may result in down-regulation, but also in up-regulation of the excitatory transmission. This apparent contradiction, however, is easily reconciled by the consideration that glutamatergic neurotransmission is essentially involved in synaptic plasticity and several of the VPA-related molecular mechanisms described below are intimately linked to promotion of plasticity itself. Furthermore, different effects of the drug may be related to the specific cellular or animal model examined and may not precisely reflect the human situation in response to therapeutic treatments. In patients, it has been observed that therapeutic doses of VPA mainly increases brain GABA levels without affecting glutamate levels [92].

VPA EFFECTS ON IONIC CHANNELS

One of the many ways through which VPA affects neural function is through modulation of ion channels, but the data are quite contrasting. Regarding voltage gated channels, VPA reduces neuronal excitability by acting on sodium as well as potassium, conductances [93]. In particular, it has been demonstrated that VPA acts on voltage-dependent sodium-channels, both by inhibiting their activity and their expression [94]. This effect can be linked to VPA-mediated protection against NMDA dependent neurotoxicity, as VPA suppresses neuronal excitability mediated by sodium currents [95-97]. On the contrary, it has been shown that chronic treatment with VPA up-regulates cell surface expression of Na^+ channels *via* transcription/translation-dependent mechanisms and this, in turn, results in the enhancement of Ca^{++} channel gating [98]. Concerning the potassium currents, VPA affects neuronal activity also by increasing the amplitude of the late potassium outward currents [99], even if the VPA effect on K^+ channels is still unclear [100]. Overall, the role of VPA regulation of ionic currents in neuroprotection is still poorly understood as, in this case too, excitability may be either downregulated or upregulated. Furthermore, no clear evidence for the contribution of ionic channel regulation emerged from experiments of neuroprotection mediated by VPA against ischemic damage in *in vitro* slices [101]. From the available data it is safe to conclude that the direct effects of VPA on voltage gated ionic channels is likely less important in neuroprotection, and possibly also in antiepileptic properties, than other mechanisms involved in the regulation of cellular pathways and that may indirectly affect voltage gated conductances.

VPA Role in Antioxidant Defense

The defense properties against oxidative stress contribute to the neuroprotective effect of VPA, as well as of lithium. Both lithium and VPA, tested on human neuroblastoma (SH-SY5Y) and glioma (SVG and U87) cells subjected to oxidative stress and endoplasmic reticulum stress, decrease the vulnerability of neural, but not glial, cells, to injury [102]. In primary cultured rat cortical cells, VPA provides neuroprotection against damage caused by oxidative stress, by inhibiting lipid peroxidation and protein oxidation [103]. Moreover, VPA regulates the expression of the three isoenzymes of glutathione S-transferase (GST M1, A3, A4), important protective factors against oxidative stress. In particular, chronic VPA treatment increases mRNA levels of M1 and A4 and decreases the A3 mRNA level, while lithium only increases levels of GST M1 mRNA and protein, thus indicating the complexity of the regulation of GST by VPA and lithium [104]. Glutathione, the major endogenous antioxidant in the brain, plays a key role in defending against oxidative damage. Chronic treatment with VPA or lithium increases both glutathione levels and the expression of glutamate-cysteine ligase, a glutathione rate-limiting synthesis enzyme, in both primary cultured rat cortical cells and neuroblastoma SH-SY5Y cell lines. Lithium and VPA inhibit hydrogen peroxide-induced cell death in primary cultured rat cortical cell through regulation of glutathione levels [105]. Moreover, chronic treatment with lithium and VPA significantly inhibits the glutamate-induced increase of intracellular free calcium, lipid peroxidation, protein oxidation, DNA fragmentation and cell death in primary cultured rat cortical cells, suggesting a precise correlation between neuroprotection and decreased oxidative burden consequent to excitotoxic insult [106]. Lithium and VPA protect against amphetamine-induced oxidative stress *in vivo* [107] and this may contribute to their role as effective drugs in the treatment of bipolar disorders. In more general terms, the promotion of antioxidant defense may be a primary mechanisms at the basis of VPA neuroprotection.

VPA Effects on Kinase Pathways

The VPA role in kinase pathways is well-established and shares both similarities and differences with the action of lithium [108]. In particular, VPA activates pro-survival protein kinases, such as the Protein Kinase B (PKB)/Akt, the Mitogen Activated Protein Kinases (MAPKs) and other kinases, such as the lipid kinase Phosphatidylinositol 3-Kinase (PI3K), while it inhibits the Glycogen Synthase Kinase 3β (GSK-3β).

The PI3K/Akt signaling pathway plays a critical role in mediating survival signals in neurons [109] and both VPA and lithium affect this pathway, as previously mentioned with reference to the "inositol-depletion hypothesis" [110, 41]. This hypothesis postulates that the therapeutic effects of lithium and VPA are exerted on phosphoinositol cycle, in ways resulting in depletion of cellular myo-inositol and consequently in dampening of phosphoinositide signaling. However, the effects of VPA and lithium on phosphoinositol cycle are quite different [111, 112], as shown in Fig. (**2**). While lithium, by inhibiting myo-inositol monophosphatase, results in depletion of inositol and concomitant accumulation of inositol 3,4,5-trisphosphate, inositol bisphosphate and inositol 1,3,4-trisphosphate, VPA has been clearly demonstrated to inhibit myo-inositol-1-phosphate (MIP) synthase [113], determining a decrease in levels of both inositol mono-phosphate and inositol. Both drugs determine inositol depletion, also by inhibiting high affinity myo-inositol transporter and down-regulating the level of its mRNA. Inhibition of inositol uptake could thus represent an additional pathway for inositol depletion due to both drugs [114]. Both lithium and VPA decrease the inositol 1,4,5-trisphosphate levels [115, 85], but while lithium acts through inhibition of inositol polyphosphatase, VPA causes an acute reduction in PIP_3 production, that may be caused either by a reduced supply of the PIP_3 precursor PIP_2 or by a direct effect on an enzyme responsible for phosphorylation of multiple phospholipid targets, such as PI3K [116]. The role of phosphatidylinositol kinases as targets for VPA has been studied, but the results are still contrasting, notwithstanding the fact that sequence variations found in one isoform of PI3K have been linked to bipolar disorder and schizophrenia [117]. While lithium blocks IP_3-dependent Ca^{++}-release, VPA has no effect on intracellular Ca^{++} level [118]. The phosphoinositol cycle is particularly important to explain the VPA effect on neuroprotection, being closely linked to the pathway of PI3K/PKB or Akt [87, 119], which plays a funda-

mental role on neuronal survival [109, 120, 121]. However, how VPA acts on the pro-survival protein kinase Akt is not clear. Similarly to lithium and to other HDACs inhibitors (sodium butyrate and TSA), VPA causes an increase in phosphorylation-dependent Akt activation, that seems to be mediated by the PI3K pathway, both in *in vitro* and *in vivo* models [119, 122-128]. However, in non neuronal cell lines, it has been demonstrated that VPA and other HDACs inhibitors could also contrast Akt1 and Akt2 expression, which leads to Akt deactivation and apoptotic cell death [129].

Other protein kinases involved in neuronal survival [130] and activated by VPA, are the MAPKs. In fact, it has been clearly demonstrated that VPA activates extracellular signal-regulated kinases (ERKs), both *in vitro* and *in vivo*, and this in turn regulates transcription factor activation and gene expression [131, 132]. An attractive hypothesis to explain the mechanism of VPA-induced MAPK activation comes from studies on endothelial [133] and mesangial cells [134]. This hypothesis suggests that VPA is incorporated into phosphatidylcholine [135] that is converted by phospholipase A2 to lysophosphatidylcholine, which in turn activates ERK through the PI3K/Janus kinase 2/MEK-1-dependent pathway. *In vitro*, VPA through the ERK pathway increases the expression of genes driven by AP-1 transcription factor, including growth cone-associated protein 43 and Bcl-2 which promote axon growth and cell survival [131]. In cultured cortical cells, VPA increases activated phospho-ERK44/42 and phospho-RSK1 (ribosomal S6 kinase 1) levels and enhances ERK pathway-dependent growth, thus promoting proliferation/maturation of neural cell precursors [136]. *In vivo*, VPA increases the levels of activated phospho-ERK44/42, activates RSK1, phospho-CREB (cAMP response element-binding protein), phospho-B cell lymphoma protein-2 antagonist of cell death and BDNF [132]. Moreover, through the ERK pathway, VPA enhances neurogenesis in the dentate gyrus of rat hippocampus [136]. Together, these data support VPA promotion of neurotrophic effects through the ERK pathway.

An opposite effect has been shown for VPA towards the Glycogen Synthase Kinase 3β (GSK-3β). This is a serine/threonine protein kinase that is implicated in multiple cellular processes, including neuronal survival, and whose dysfunction is linked to the pathogenesis of several brain diseases. GSK-3β is regulated through phosphorylation by several kinases, such as Akt, MAPKs, PKC and PKA. GSK-3β plays a critical role in the CNS, by regulating various cytoskeletal proteins, as well as long-term nuclear events, and is a common target for lithium and VPA, which both inhibit its activity [137-139]. While lithium directly inhibits GSK-3β, independently of its phosphorylation state, the ability of VPA to directly act on GSK-3β is still debated. However, both agents increase the inhibitory phosphorylation of GSK3β, even if in different ways [122, 140]. In human neuroblastoma cells, VPA concentration-dependently inhibits GSK-3β, thus resulting in significant time-dependent increase in both cytosolic and nuclear levels of β-catenin, a marker of WNT signaling [141]. In cerebellar granule neurons (CGNs), VPA affects axonal morphology, by inhibiting the microtubule associated protein (MAP)-1B GSK-3β-mediated phosphorylation [142]. This VPA-dependent GSK-3β downregulation protects CGNs against neurotoxicity of platelet activating factor (PAF), produced from brain macrophages infected with human immunodeficiency virus type 1 (HIV-1) [143]. *In vivo*, the results about the VPA effect on GSK-3β are contrasting. Chronic VPA treatment, at therapeutically relevant doses, changes neither GSK-3β activity nor its expression in rat frontal cortex [144], while it affects levels of β-catenin, an established marker of GSK-3β inactivation [145]. However, VPA counteracts the pathophysiological serine-dephosphorylation of GSK-3β caused by hypoxia in mouse brain *in vivo* [146].

Both lithium and VPA inhibit protein kinase C (PKC) activity, after sub-chronic administration, in cell culture as well as *in vivo* [147]. VPA decreases PKC activity and the expression of both PKC alpha and epsilon subunits [148], as well as the expression of a

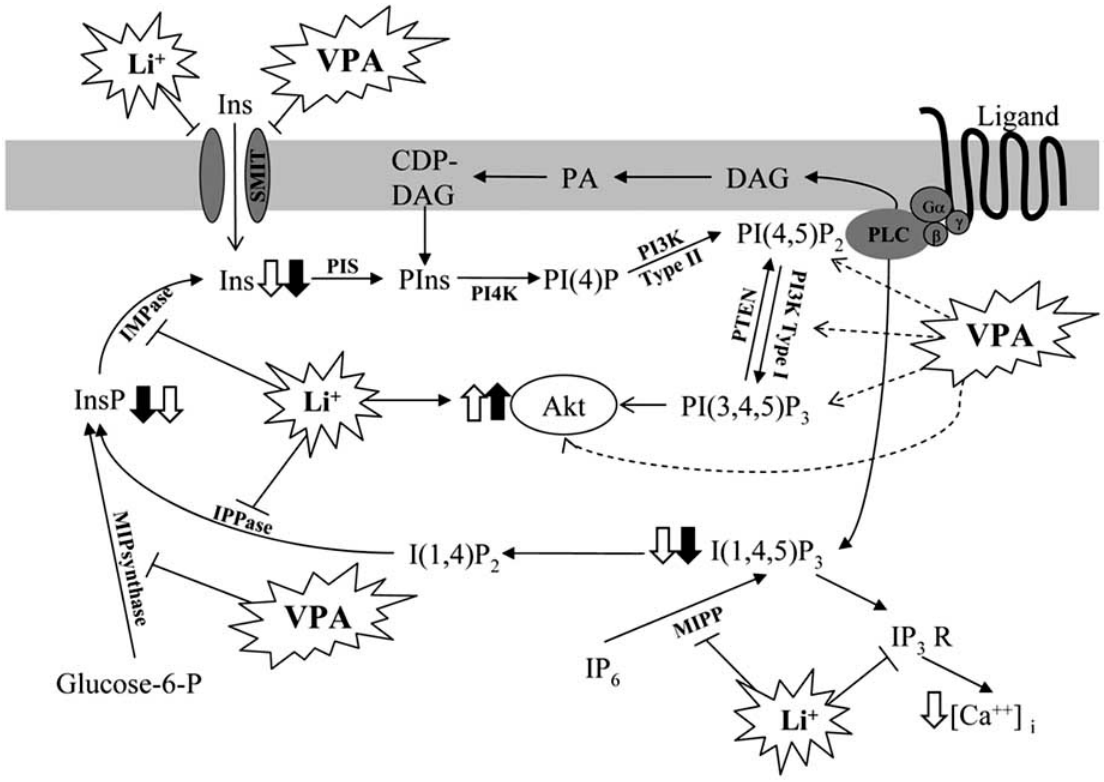

Fig. (2). Scheme of the lithium and VPA effect on inositol metabolic pathway. The bold arrows (white for Li^+ and black for VPA) down indicate a decrease, while the bold arrows up mean an increase in the intracellular levels of the different inositol species or of Akt activation. The continuous thin lines indicate the Li^+ effect, while the dashed lines the VPA ones. For both, ⊥ means inhibition, while the arrow end indicates activation.

major PKC substrate, MARCKS, implicated, in developmental and adult neural plasticity. This leads to a downstream impact on GSK-3β and on the AP-1 family of transcription factors [149-151]. In addition to MARCKS, another plasticity-related PKC substrate, GAP-43, has been identified as a target of VPA which reduces MARCKS and increases GAP-43 expression through PKC inhibition [152]. *In vivo*, both lithium and VPA prevent working memory deficits by decreasing PKCα phosphorylation-dependent activity [153].

The effect of VPA on PKA activity is debated. It has been demonstrated that VPA decreases stimulated cAMP production in glioma, but not in neuroblastoma cells [154, 155]. In rat cortical neurons, treatment with VPA does not affect basal cAMP levels but significantly inhibits forskolin-stimulated cAMP increase [156].

Substrate phosphorylation through kinases is a common intermediate step of many cascades regulating main cellular function. The involvement of VPA in so many and so important mechanisms regulated by cellular kinases underlies the enormous potential of this drug to interfere with fundamental mechanisms of cell function or dysfunction. Main cellular pathways affected by VPA and discussed above are summarized in Fig. (**3**).

VPA Effects on Gene Expression

Valproic acid has been demonstrated to exert its effects on gene expression regulation through two main mechanisms: on one side VPA regulates gene expression by acting on transcription factors, mainly through phosphorylation regulation, while on the other side VPA, as a HDACs inhibitor, affects epigenetic modulation of gene expression.

Effects on Transcription Factors

The fact that VPA clinically requires a lag period for the onset of its effects and that these effects are not immediately reversed upon discontinuation of treatment suggests that it acts at the level of gene expression, by regulating the activity of key transcription factors. Among them, is the transcription factor Activator Protein-1 (AP-1), which consists of a variety of dimers formed by members of the Jun and Fos families of proteins. AP-1 has been shown to affect several brain functions, such as development, plasticity and neurodegeneration [157]. VPA-treatment of rat C6 glioma cells, concentration- and time-dependently increases AP-1 DNA binding activity [158]. In human neuroblastoma SH-SY5Y cells, at therapeutically relevant concentrations, both lithium and VPA acutely (<24 h) induce c-Fos immunoreactivity and AP-1 binding, while chronic (1 week) treatment with VPA leads to continued induction of c-Fos, c-Jun and FRA (Fos-like Region Antigens) resulting in AP-1 DNA binding activity, an effect not replicated by lithium [159]. In both cell lines, VPA robustly enhances AP-1 mediated gene expression [160]. Considering that phosphorylation of c-Jun by GSK-3β inhibits AP-1 DNA binding activity, both VPA and lithium seem to activate AP-1 trough GSK-3β inhibition [160]. C-Jun regulation through phosphorylation is a very complex phe-

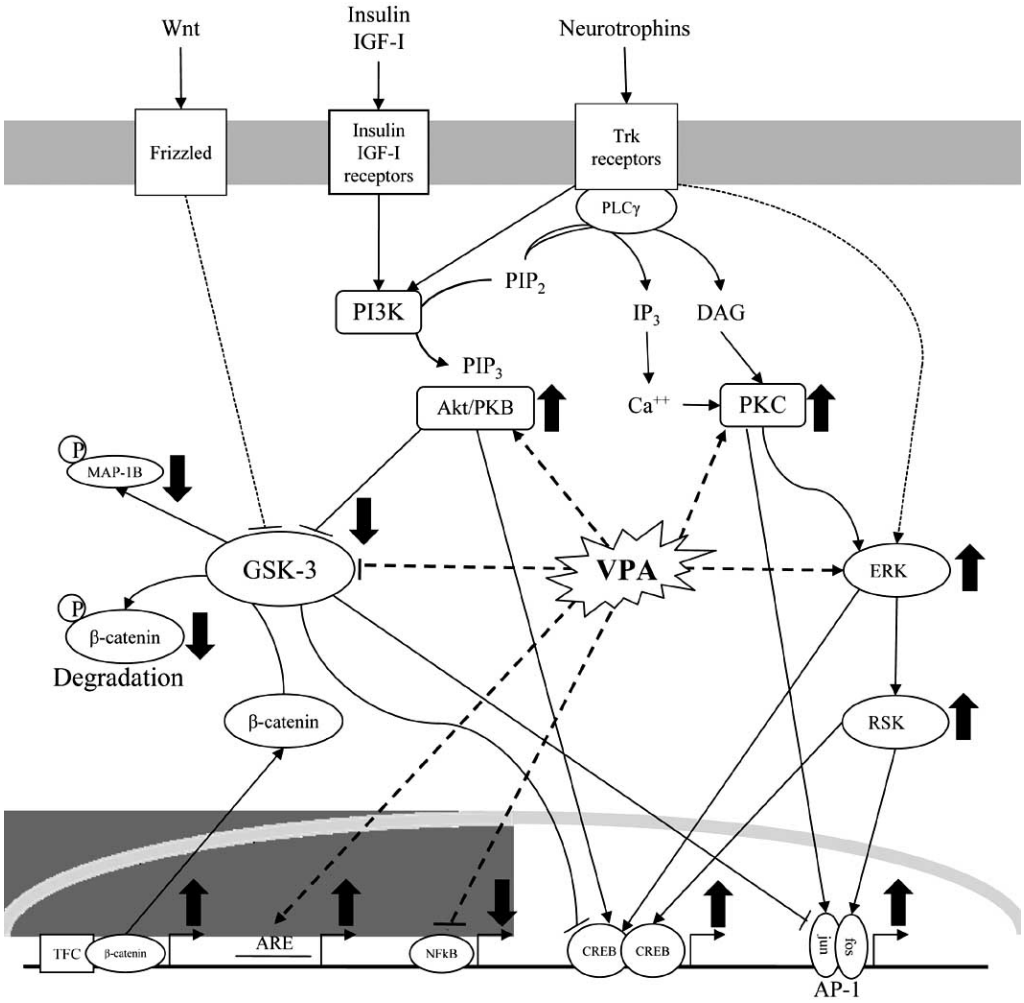

Fig. (3). Scheme of the intracellular protein kinase pathways affected by VPA. The dashed lines indicate the pathways positively (→) or negatively (⊥) regulated by VPA. The bold arrows indicate the increasing (up) or decreasing (down) VPA effect on the enzymatic or transcriptional activities.

nomenon. To become active c-Jun needs to be both dephosphorylated at its C-term and phosphorylated at its N-term [161] GSK-3β phosphorylates the C-terminal regions of Jun family transcription factors at sites proximal to their DNA binding domains, thus decreasing their affinity for AP1 consensus sequences and their transactivating potential [162]. Considering that inhibition of GSK-3β and dephosphorylation of the GSK-3β sites in the Jun family members increases their transactivating potential, VPA-dependent GSK-3β inhibition could be proposed as the possible mechanism of AP-1 transactivation by the drug. The VPA effect on AP-1 determines transactivation of several genes, including GAP-43, Bcl-2 and serotonin-2A (5-HT2A) receptor [131, 163]. It has also been demonstrated that VPA induces transcription of the Galpha(i2) gene promoter, which lacks AP-1-binding motifs. This is because the gene expression-inducing effect of VPA occurs, depending on its HDAC inhibition activity, may be also exerted through the Sp family of transcription factors [164, 165], as well as the redox-sensitive transcription factor that interact with the Antioxidant Response Element (ARE). Accordingly, by using two well-characterized ARE-driven gene promoters, i.e., mouse heme oxygenase-1 and human NAD(P)H:quinone oxidoreductase 1 genes, it has been observed that VPA-induced gene transcription is abrogated by antioxidants [166]. Another transcription factor regulated by VPA is NF-kB, which is inhibited in glioma cells with the result of decreasing TNF-alpha and IL-6 expression and thus modulating immune response [167]. The VPA effect on CREB, a bZIP transcription factor that is activated through phosphorylation and is involved in a wide-range of brain processes, including development, synaptic plasticity and memory [168], is still debated. In glioma cells, VPA does not affect CREB DNA binding activity [163]. In human neuroblastoma SH-SY5Y cells chronic, but not acute treatment with lithium chloride inhibits phosphorylation and DNA binding of CREB induced by the adenylate cyclase activator forskolin, without any direct effect on CREB expression [155]. In PC12 neurons, VPA regulates tyrosine hydroxylase gene expression through cAMP/CREB-dependent pathway [169].

In vivo contrasting effects have been reported too. Chronic VPA significantly increases the AP-1 DNA binding activity and decreases the one mediated by NF-kB in frontal cortex, while it does not alter CREB binding activity [170]. In another study, however, chronic VPA at therapeutically relevant concentrations is reported to increase the level of CREB phosphorylation and, therefore, BDNF expression in the rat cortex and hippocampus through ERK pathway [132]. Regarding CREB, contrasting results in the same brain areas have been reported in a different study [171]. From these data, it seems that VPA exerts differential effects depending on the brain region examined, the duration and the dose of treatment. To render the issue further intriguing, acute VPA increases the density of p-CREB-positive cells and enhances p-CREB, but not p-ERK, protein levels in the amygdala and the accumbens. In contrast, following chronic VPA, p-CREB and p-ERK protein levels are markedly attenuated in the amygdala, while the number of p-CREB immunoreactive cells is increased in the accumbens [172].

Effects on Histone Deacetylases

While lithium and VPA both regulate gene expression trough transcription factors [173], VPA can also regulate gene expression through a distinct mechanism that involves epigenetic regulation of transcription. In fact, VPA efficiently inhibits HDACs, negative regulators of gene expression in multiple settings, at therapeutically relevant levels [22]. Moreover, VPA alters chromatin structure through negative regulation of chromatin associated proteins, such as several members of the Structural Maintenance of Chromatin (SMC) proteins, SMC-associated proteins, DNA methyltransferase and heterochromatin proteins. Down-regulation of these proteins is associated with chromatin decondensation and correlates with enhanced sensitivity of DNA to nucleases. Modulation of heterochromatin proteins is not a direct, but a downstream, effect of histone acetylation [174]. VPA inhibits HDACs activity, most likely by binding to the catalytic center of the enzyme [21]. VPA, in addition to selectively inhibiting the catalytic activity of class I HDACs, induces proteasomal degradation of HDAC2, in contrast to other HDAC inhibitors such as trichostatin A (TSA) [175]. Comparison of VPA to other HDACs inhibitors, such as TSA, has been often used to confirm the fact that a VPA effect was due to its HDACs inhibitory activity and not to other VPA side actions. In several cell lines, it has been observed that TSA and VPA induce expression of the endogenous reelin promoter, TSA being considerably more efficient [176]. *In vivo*, VPA reverts down-regulation of reelin and GAD67 in both WT and heterozygous reeler mice [75]. This result is very interesting to explain the VPA effects on mood disorders, as down-regulation of reelin and GAD67 expression is well-documented in psychiatric patients. In addition, VPA effectively increases the binding of acetyl-histone H3 to RELN and GAD67 promoters, suggesting that histone H3 covalent modifications modulate DNA demethylation in terminally differentiated neurons and supporting the view that, either directly or indirectly, VPA may facilitate DNA demethylation through HDACs inhibition [76]. This data is supported by the fact that VPA triggers replication-independent active demethylation of DNA in non-dividing cells, because it reverses DNA methylation patterns and erases stable methylation imprints on DNA independently on its replication. This also strengthens the concept that chromatin acetylation and DNA methylation are found in a dynamic interrelation and that the consequences of HDAC inhibitors are not limited to changes in histone acetylation, but that they also bring about a change in DNA state, especially in its methylation [177, 178].

The fact that VPA regulates genes involved in neuronal survival/death through its HDACs inhibitory activity has been shown both in *in vitro* and *in vivo* models. For example, several HDACs inhibitors, including VPA, by acting on class I HDACs, HDAC1 and 3, dramatically decrease the protein level of the transcription factor Hypoxia Inducing Factor (HIF)-1alpha and its transcriptional activity in cell lines [179]. The VPA effect as HDACs inhibitor could be also responsible for the increased neurotrophins expression, such as BDNF and GDNF, related to both neuroprotective and memory-enhancer properties of this drug [180-182]. Moreover, in neuronal cultures, VPA increases the expression of 5-lipoxygenase (5-LOX) and, *in vivo*, VPA treatment increases the acetylation of histone H3 and the 5-LOX content in mouse hippocampus [183]. VPA has been shown to induce differentiation and cell death of neuroblastoma cells, possibly associated with its HDACs inhibiting activity and with an activated Notch signaling cascade, as shown by increased levels of intracellular Notch-1 and Hes-1 [184]. In human neuroblastoma cells, p21(Cip1) is a target of induction of transcription and protein expression for both lithium and VPA, while only VPA induces a concomitant increase of p27(Kip1) gene expression [185]. Similarly, in medulloblastoma cells, VPA determines induction of histone (H3 and H4) hyperacetylation, activation of p21, and suppression of TP53, CDK4, and CMYC expression, with a strong correlation between induction of histone hyper-acetylation and regulation of pathways critical for maintaining growth inhibition and cell cycle arrest [186]. Taken together, all these developmental effects of VPA lead to associate VPA-induced teratogenesis with histone deacetylase inhibition *in vivo*, possibly explaining why VPA causes spina bifida if taken during pregnancy [187]. The VPA effect on HDACs up-regulates neuron-specific genes through a neuron-located basic helix-loop-helix transcription factor, NeuroD, [188] and it increases expression of cyclin D2 as well as of survival genes, such as Bad and Bcl-2, leading to enhancement of GABAergic neuron number, neurite outgrowth and neuronal differentiation [189]. *In vivo*, neuroprotection against malonate toxicity in rat striatum correlates with an increase in histone acetylation and a decrease of heat-shock protein 70 (HSP-70) and fos expression, while the levels of bcl-2 are unaffected [91]. VPA also prevents the reduction in levels of acetylated histone H3 in the ischemic brain,

induces HSP70 and blocks ischemia-induced down-regulation of phospho-Akt, as well as ischemia-elicited up-regulation of p53, inducible nitric oxide synthase and cyclooxygenase-2 [124]. In addition, VPA alleviates cerebral inflammation and perihematomal cell death after intracerebral hemorrhage through several mechanisms mediated by transcriptional activation following HDACs inhibition. In fact, by increasing histone H3 acetylation as well as the translation and the post-translational modifications of pro-survival proteins, such as pERK, pAKT, pCREB and HSP70, VPA up-regulates the expression of anti-apoptotic genes, such as bcl-2 and bcl-xL, while it down-regulates the expression of a pro-apoptotic gene, like bax. Moreover, VPA exerts an anti-inflammatory action through the down-regulation of inflammatory genes, mainly Fas-L, IL-6, MMP-9, MIP-1, MCP-1, and tPA [190]. VPA potently blocked seizure-induced neurogenesis, an effect that appeared to be mainly mediated by inhibiting HDACs and normalizing HDAC-dependent gene expression within the epileptic dentate area, and it protected the animals from seizure-induced cognitive impairment in a hippocampus-dependent learning task [191]. The VPA effect on histone acetylation has been examined also in a clinical setting, including schizophrenic and bipolar patients, in which ac-H3 and ac-H4 levels from lymphocyte nuclear protein extracts resulted significantly increase by VPA treatment [192]. Main information on genes regulated through VPA-mediated mechanisms is summarized in Table **1**.

VALPROIC ACID EFFECTS ON NEUROGENESIS, NEURONAL DIFFERENTIATION AND SURVIVAL

Neurogenesis in the adult is a multistep process that includes proliferation of precursor cells, migration of the newborn cells, differentiation, integration into neuronal circuits, and survival. Both *in vitro* and *in vivo* studies have demonstrated a potent modulatory action of VPA on neurogenesis. In neuroblastoma cell lines and in cultured cortical neurons as well as *in vivo* in the dentate gyrus of the hippocampus, VPA regulates neurogenesis, neurite outgrowth, and neuronal survival, by activating the ERK pathway and by inducing its neurotrophic actions [131, 136]. In SH-SY 5Y cells, VPA promotes neurite outgrowth and prevents cell death, but it has no effect on cell proliferation. This neurotrophic effect does not involve inhibition of GSK-3β, HDACs or PKC, but VPA activation of ERK/MAPK signaling pathway [193]. In fact, VPA, as previously described, affects the functions of GSK-3β and HDACs, but its effects on the ERK pathway are not fully mimicked by selective inhibitors of GSK-3β or HDACs [136]. However, inhibition of GSK-3β mimics WNT-7a signaling, by inducing axonal remodeling and clustering of synapsin I in developing neurons [142]. It is possible that VPA stimulates synthesis and secretion of neurotrophic factors, which in turn activate the ERK pathway. Significant increases in BDNF and GDNF mRNA expression are found in a glioblastoma cell lines and in primary astrocytes following VPA treatment and these effects may play a major role in mediating VPA-induced neuroprotection [180]. Hippocampal HN33 cells exposed to VPA exhibit reduced MARCKS protein expression due to alteration in PKC activity and demonstrate increased GAP-43 protein expression with concomitant alterations in cellular morphology, including an increase in the number and length of neurites and a reduction in cell growth rate [152]. Recently, it has been demonstrated that VPA induces the expression of neurotrophic genes, such as TrkB, BDNF, MnSoD, and NeuroD, in neural stem cells from an animal model of the Niemann–Pick type C disease, the NPC1-deficient mice [194]. This promotes neuronal differentiation, but not astrocyte differentiation through a mechanism involving VPA ability to act as an HDACs inhibitor. The HDACs inhibition by VPA treatment decreases the REST/NRSF gene, which normally acts as a negative regulator of the expressions of many neuron-specific genes. In a model system of adult hippocampal multipotent neural progenitor cells, the presence of VPA reduces their proliferation and promotes their differentiation into neurons. In this model, analysis of the mechanism of VPA-mediated neuronal differentiation is based on up-regulation of the neurogenic bHLH transcription factor NeuroD, due to HDACs inhibition [188]. Among the many genes involved in survival and differentiation of neurons and regulated in their expression by VPA, it is relevant to recall that the drug also activates BDNF promoter IV through inhibition of HDACs [195]. In some models, VPA promotes neuronal differentiation to the detriment of astrocyte and oligodendrocyte differentiation [196, 197]. In embryonic rat cortical or striatal primordial stem cells, VPA increases the number and percentage of neurons, especially GABAergic neurons, stimulates neurite out-

Table 1. Genes Affected by VPA Through Transcription Factors

VPA-induced Transcriptional Activation			
Transcription Factor	**Neuronal Cell Line or Primary Culture**	**Gene**	**Reference**
AP-1	SH-SY5Y neurons	GAP-43	[131]
"	"	Bcl-2	[131]
"	C6 Glioma cells	5HT-2A receptor	[163]
Sp1	K562 cells	Galpha(i2)	[164]
"	A673 neurons	neuronal nitric oxide synthase	[251]
CREB	PC-12	tyrosine hydroxylase	[169]
"	"	"	[252]
"	Rat hippocampus and cortex	BDNF	[132]
NeuroD	hippocampal neural progenitor cells	Synapsin I	[189]
"	"	SCG 10	[189]
VPA-induced Transcriptional Repression			
Transcription Factor	**Neuronal Cell Line or Primary Culture**	**Gene**	**Reference**
NF-kB	A-172 glioma cells	TNF-alpha	[167]
"	"	IL-6	[167]
"	Rat cortex	COX-2	[170]

growth, increases neuronal differentiation and decreases the number of astrocytes, without changing the total number of cells [189, 196]. This effect of VPA involves an increase of cyclin D2, which promotes the progression from G1 to S phase, and of prostaglandin E2 synthase. In the nervous system, prostaglandin E2 (PGE2), produced by PGE synthase, mediates different neurophysiological responses [198]. In addition, considering that histone modifications affect timing of oligodendrocyte progenitor differentiation in the developing rat brain, administration of VPA results in significant hypomyelination with delayed expression of late differentiation markers and retained expression of progenitor markers in the developing corpus callosum [197]. VPA induces differentiation and cell death of neuroblastoma and PC12 cells, possibly associated with its HDACs inhibiting activity [184, 199]. VPA stimulation of neuroblastoma cell lines increases cell death and phenotypic changes associated with differentiation, that is neurite extension and up-regulation of neuronal markers. As previously mentioned, this treatment also activates Notch signaling cascade, thus mimicking the initial phase of induced differentiation [184]. In N1E-115 neuroblastoma cells, VPA up-regulates Gadd45a to trigger activation of the downstream JNK cascade controlling neurite outgrowth [200]. Moreover, VPA inhibits the collapse of sensory neuron growth cones and increases growth cone area. These effects do not depend on GSK-3β or HDACs inhibition, but on inositol depletion [173].

VALPROIC ACID EFFECTS ON NEURODEGENERATION AND NEUROPROTECTION

VPA Effects on Neurotoxicity In Vitro

The neuroprotective effect of VPA has been demonstrated in several neuronal models *in vitro*. However, under some conditions,VPA can even exacerbate neuronal death, instead of being neuroprotective [201]. Similarly to lithium, VPA protects CGCs from apoptosis induced by low potassium, but through different kinase signaling pathways [87]. However, while lithium protects cultured CGCs from GSK-3β-mediated apoptosis induced by trophic factor withdrawal by preventing c-jun induction, VPA does not provide any neuroprotection and potentiates both c-jun expression and cell death [201]. The fact that neuroprotection mediated by lithium and VPA may be, at least in part, due to activation of different pathways suggests that a combination of the drugs may better work in some cases. Recent evidence for synergistic neuroprotective effect of lithium through GSK-3 inhibition and of VPA through HDACs inhibition, is promising [202]. VPA protects mature CGCs from excitotoxicity induced by SYM 2081 ((2S, 4R)-4-methylglutamate), an inhibitor of excitatory amino-acid transporters, and by an agonist of low-affinity kainate receptors. SYM-induced apoptosis is associated with a rapid and robust nuclear accumulation of the pro-apoptotic gene, GAPDH. VPA treatment suppresses SYM-induced GAPDH nuclear accumulation, concurrent with its neuroprotective effects, by acting on histone H3 acetylation [203]. In CGCs, VPA prolongs life span of these neurons, when added to maturing culture,s associated with a robust increase in histone H3 acetylation levels, a protective effect mimicked by treatment with another HDACs inhibitor, TSA, but not by VPA analogs, which are inactive in blocking HDACs [204]. Moreover, in cultured cerebral cortical neurons, both VPA and lithium are neuroprotective against excitotoxicity. The neuroprotective effects of lithium involve inhibition of NMDA receptor-mediated calcium influx, while the mechanism of VPA action is less clear [205], but it seems also to be linked to oxidative stress [106]. VPA protects cultured rat hippocampal neurons against amyloid beta- and glutamate-induced injury, by stabilizing intracellular calcium concentration and counteracting alterations in tau and ubiquitin [206]. Both *in vitro* and *in vivo*, VPA protects against glutamate-induced excitotoxicity through HDACs inhibition and the consequent endogenous alpha-synuclein induction [196]. We have shown that VPA protects CGCs in culture from 6-OHDA neurodegeneration, by using a similar molecular mechanism [207]. In a model of human immunodeficiency virus-1 encephalitis, VPA treatment of cortical neurons exposed to HIV-1 gp120 prevents neurotoxicity through induction of neurite outgrowth. Similarly, VPA protects Severe Combined Immuno-Deficient (SCID) mice against the neurodegeneration of HIV-1ADA infected Monocyte-Derived Macrophages (MDMs), possibly through its effects on the phosphorylation of tau and beta-catenin [208]. In neuroblastoma cell lines, VPA attenuates rotenone (a potent complex I inhibitor)-induced apoptosis through the induction of heat shock protein 70, which may interact with the apoptotic Protease-Activating Factor 1 (PAF-1). Activation through phosphorylation of the pro-survival proteins Akt, Bcl-2, as well as Erk1/2, by VPA contributes to the protection [123]. In corticostriatal brain-slices, VPA protects from simulated ischemia [101] and in hippocampal slice cultures from death induced by oxygen/glucose deprivation [209], while in organotypic slice culture of mouse spinal cord, VPA protects spinal motoneurons against death from glutamate toxicity [210]. By using primary neuron-glia cultures from midbrain, VPA results to be a potent neuroprotective agent against LPS-induced neurotoxicity, by decreasing levels of pro-inflammatory factors released from activated microglia [211]. Microglia are intrinsic immune effector cells of the brain. Their activation has been increasingly associated with pathological development of several neurodegenerative diseases, including Alzheimer's disease, AIDS dementia, Parkinson's disease and multiple sclerosis. While activation of microglial cells may be intended primarily to protect neurons, frequently the uncontrolled production of inflammatory factors derived from them results in neuronal loss [212]. It is, therefore, interesting that VPA causes a decrease in the number of microglia due to an apoptotic mechanism [213]. Accordingly to these data, VPA also induces apoptosis in a microglial cell line (BV-2) through a caspase 3-mediated action [214]. The VPA induced apoptosis of microglia cells is mainly due to the HDACs inhibition, as demonstrated by the fact that also TSA and sodium butyrate (SB), two structurally dissimilar HDACs inhibitors, induce microglial apoptosis [213].

VPA Effects on *In Vivo* Models of Neurodegeneration

VPA has been used for several years in the treatment of epilepsy [215]. Epileptogenesis, the process leading to epilepsy with spontaneous recurrent seizures, can be initiated by a number of brain damaging insults, including traumatic brain injury, status epilepticus and stroke, and it is often associated with memory impairment and behavioral problems. There has been a growing interest in the use of antiepileptic drugs, especially VPA, for neuroprotection and prevention of such brain insults. VPA is neuroprotective after seizures induced by several treatments, such as beta-vinyllactic acid, quisqualate, kainate and kindling [216-219]. In particular, VPA reduces brain damage and improves functional outcome in a rat model of epilepsy due to electrical stimulation of basal amygdala [220]. In addition, in a rat permanent middle cerebral artery occlusion (pMCAO) model, post-pMCAO injections of HDAC inhibitors, VPA, SB or TSA decrease brain infarct volume. In a similar model, post-insult treatment with VPA protects from ischemic damage by inhibiting HDACs and inducing HSP70 [221]. VPA neuroprotection has been tested in several models of chronic neurodegenerative diseases that are often characterized by cognitive deficits, as VPA also exerts a memory-enhancer effect [181, 222, 223]. VPA has been used to treat behavioral disturbances in Alzheimer's disease since early '90s [224], but it later emerged also the possible neuroprotective effect of VPA in this pathology, considering its effect on apoptosis inhibition and on slowing down the formation of neurofibrillary tangles. Clinical trials are now on-going to examine whether chronic valproate therapy can attenuate the clinical progression of AD [225, 226]. To support this hypothesis, it has been recently demonstrated that VPA, as well as lithium, inhibits beta-amyloid peptide production in cell lines stably transfected with

the Swedish mutation of amyloid precursor protein a well as in the brains of the PDAPP (APP(V717F) Alzheimer's disease transgenic mouse model, by acting on GSK-3β [227].

In view of the biochemical evidence of decreased GABAergic function in Parkinson's disease and of the above-described effects of VPA on GABAergic transmission, the study of the VPA effect on Parkinson's disease started in the '70s, but no amelioration was noticed in patients [228-230]. However, it has been shown that in neuroblastoma cell lines VPA attenuates apoptosis induced by rotenone, a potent complex I inhibitor, which induces a Parkinson-like neuronal death, and that VPA pretreatment protects cultured midbrain dopamine neurons from MPP^+-induced neurotoxicity, another model of PD-like neurodegeneration [123, 231]. Similarly to PD, VPA has been tried since the '70s for the treatment of Huntington's disease (HD) with no relevant results in patients [232, 233]. However, in animal models of HD, VPA restored the normal levels of GABA [234]. More recently, it has been shown that several HDACs inhibitors ameliorate motor deficits in mouse models of HD [235, 236], thus supporting the possible role of VPA for HD treatment [237].

In a transgenic mouse model of ALS expressing the G86R mutant SOD1, histone deacetylase inhibition through VPA treatment restores proper acetylation levels and displays an efficient neuroprotective capacity against oxidative stress *in vitro*. Moreover, when injected *in vivo*, the VPA maintains normal acetylation levels in the spinal cord and significantly prevents motoneuronal death in these animals [238]. However, despite neuroprotection, the survival of treated animals was not significantly increased [238], contrary to a previous report [210]. More recently, it has been observed that combined treatment with lithium and VPA produces a greater and more consistent effect in delaying the onset of disease symptoms, prolonging the lifespan and decreasing the neurological deficit scores, compared with the results of mono-treatment with either lithium or VPA, mainly by acting on GSK-3β [239].

VPA is under clinical investigation for the treatment of spinal muscular atrophy (SMA), an alpha-motoneuron disorder caused by insufficient expression of Survival Motor Neuron (SMN) proteins [240, 241]. Homozygous absence of SMN1 gene is the primary cause of SMA, whose severity is mainly determined by the number of SMN2 copies, which in turn depends from the correct splicing of the SMN2 gene. In cultured fibroblasts derived from SMA patients treated with therapeutic doses of VPA, the level of full-length SMN2 mRNA/protein increases and this up-regulation of SMN is most likely attributed to the correct splicing of SMN2 RNA as well as to an SMN gene transcription activation. Moreover, VPA is also able to increase SMN protein levels through transcription activation in rat organotypic hippocampal brain slices and it also increases the expression of splicing proteins, which may have important implications for other disorders due to alternative splicing [242]. In type I SMA patient-derived fibroblast cell lines, VPA increases levels of exon 7-containing SMN transcript and SMN protein, both by activating the SMN promoter and by preventing exon 7 skipping in SMN transcripts [243]. In both cultured neurons and tissues, histone acetylation modulates SMN gene expression and pharmacological manipulation through VPA of this epigenetic determinant is feasible. HDAC2, in particular, may be a future therapeutic target for SMA [244]. Altogether, these results indicate VPA as a potential treatment for SMA.

While some of the results obtained in animal models are promising, the relatively few available data from VPA-based clinical trials are less encouraging. This is not unusual when one pass from animal experimentation to patient treatment and the reasons may be multiple. In the case of VPA, some of the negative results may be linked to the necessity to use high dosages of the drug over long time span. It is hoped that second generation VPA-based drugs may bring to better results [2], together with an expansion of the cohorts enrolled in the clinical trials.

CONCLUSIVE REMARKS

The present review highlights the tremendous amount of molecular interactions and cellular processes affected by VPA. A single molecule, whose biological activities span from interactions with receptors and channels to regulation of so many catalytic reactions and from a central role in the main cellular cascades to profound regulation of gene expression, is clearly a tool that may stimulate the interest of a broad range of researchers, from pharmacologists and molecular biologists to physiologists and clinicians. This explains why, in the course of the last two decades, the interest on VPA has shifted from its use in some pathological states, most notably epilepsy and mood disorders, to such apparently unrelated issues as influencing memory in normal function and cognitive disorders, affecting neurogenesis and contrasting cell proliferation rates in tumor cells. The fact that VPA is an HDACs inhibitor largely explains the VPA effect in a wide range of neuropathologies. In fact, during the last years, it clearly emerged that alterations of epigenetic mechanisms are involved in the pathogenesis of several diseases, especially complex brain diseases [245]. Indeed, it has been demonstrated that not only genetic diseases with single-gene mutations affecting chromatin remodeling, such as Rubinstein-Taybi syndrome and Rett syndrome, but also multifactor psychiatric diseases, mainly depression and schizophrenia, are characterized by epigenetic alterations of gene expression [246, 247]. Moreover, it is emerging that alterations of epigenetic mechanisms are implicated in both neuronal death and cognitive defects [248, 249]. Therefore, HDACs inhibitors, by positively affecting both neuronal degeneration and cognitive deficits, seem to be very promising drugs against neurodegenerative diseases [250]. Among the HDACs inhibitors, VPA is one of the most interesting drug, as its HDAC inhibitor action is accompanied by a wide range of biochemical and molecular effects, which all appear to cooperate in neuroprotection and cognitive enhancement. Of course, these very exciting perspectives must be confronted for safety purposes with the potential drawbacks of such a multi-acting drug, as VPA. In every future research, therefore, it will be very important to evaluate, in parallel with the study of the beneficial effects of VPA in the various models, unwanted side effects and interactions with other drugs, whenever possible.

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ABBREVIATIONS

5-HT	=	Serotonin
5-LOX	=	5-lipoxygenase
6-OHDA	=	6-hydroxydopamine
AD	=	Alzheimer's Disease
ALS	=	Amyotrophic Lateral Sclerosis
AP-1	=	Activator Protein-1
ARE	=	Antioxidant Response Element
BDNF	=	Brain Derived Neurotrophic Factor
bHLH	=	Basic Helix-Loop-Helix
CAMK-II	=	Calcium/calmodulin-dependent protein kinase II
cAMP	=	cyclic Adenosine Mono Phosphate
CDK4	=	Cyclin Dependent Kinase 4
CGNs	=	Cerebellar Granule Neurons

CREB = cAMP Response Element-Binding protein
EAAC-1 = Excitatory amino acid carrier-1
ERKs = Extracellular signal-regulated kinases
Fas-L = Fas-Ligand
FDA = Food and Drug Administration
FRA = Fos-like Region Antigens
GABA = Gamma aminobutyric acid
GAD = Glutamate decarboxylase
Gadd45 = Growth Arrest and DNA Damage 45
GAP-43 = Growth Associated Protein-43
GAPDH = GlycerAldehyde 3-Phosphate DeHydrogenase
GLAST = Glutamate-aspartate transporter
GLT-1 = Glial glutamate transporter-1
GluR1 = Glutamate receptor subunit 1
GSK-3β = Glycogen Synthase Kinase 3β
GST = Glutathione S-transferase
HD = Huntington's disease
HDAC = Histone deacetylase
HIF = Hypoxia Inducing Factor
HIV-1 = Human Immunodeficiency Virus type 1
HSP = Heat-Shock Protein 70
I(1,4)P2 = Inositol (1,4) bis-phosphate
I(1,4,5)P3 = Inositol (1,4,5) tris-phosphate
IL-6 = InterLeukin-6
IMPase = Inositol monophosphatase
InsP = Inositol mono-phosphate
IP3 = Inositol tris-phosphate
IP3Rs = Ryanodinic inositol tris-phosphate receptors
IP6 = Inositol hexa-phosphate
IPPase = Inositol poly-phosphate phosphatase
JNK = c-Jun N-terminal Kinase
LPS = LipoPolySaccharide
MAP-1B = Microtubule Associated Protein-1B
MAPKs = Mitogen Activated Protein Kinases
MARCKS = Myristoylated Alanine-Rich C Kinase Substrate
MCP-1 = Monocyte Chemotactic Protein-1
MDMs = Monocyte-Derived Macrophages
MIP synthase = Myo-inositol-1-phosphate synthase
MIP-1 = Macrophage Inflammatory Protein-1
MIPP = Multiple inositol poly-phosphate phosphatase
MMP-9 = Matrix MetalloPeptidase-9
MPP^+ = 1-methyl-4-phenylpyridinium
MS = Multiple Sclerosis
NF-kB = Nuclear Factor- kB
NR2A = NMDA receptor 2A
NR2B = NMDA receptor 2B
PAF = Platelet Activating Factor
PAF-1 = Protease-Activating Factor-1
PD = Parkinson's Disease
PGE_2 = ProstaGlandin E2
$PI(4,5)P_2$ = Inositol (4,5) bis-phosphate
PI3K = Phosphatidylinositol 3-Kinase
PIP_2 = Phosphatidil inositol bis-phosphate
PIP_3 = Phosphatidil inositol tris-phosphate
PKA = Protein Kinase A
PKB = Protein Kinase B
PKC = Protein Kinase C
pMCAO = Permanent Middle Cerebral Artery Occlusion
REST/NRSF = Repressor Element Silencing Transcription Factor/Neuron-restrictive Silencing Factor
RSK = Ribosomal S6 Kinase
SB = Sodium Butyrate
SCID = Combined Immuno-Deficient
SMA = Spinal Muscular Atrophy
SMC = Structural Maintenance of Chromatin
SMITs = Myo-inositol transporters
SMN = Survival Motor Neuron
SOD = SuperOxide Dismutase
SSA-DH = Succinate semialdehyde dehydrogenase
TNF-alpha = Tumor Necrosis Factors-alpha
TP53 = Tumor Protein 53
tPA = Tissue Plasminogen Activator
TSA = Trichostatin A
VPA = Valproic acid

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