The Vitamin D Neuroendocrine System as a Target for Novel Neurotropic Drugs

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Abstract: Vitamin D is a seco-steroid hormone with multiple functions in the nervous system. Physiological brain mechanisms of vitamin D and its receptors include neuroprotection, antiepileptic effects, immunomodulation, possible interplay with several brain neurotransmitter systems and hormones, as well as the regulation of behaviours. Here we review the important role of the vitamin D neuroendocrine system in the brain, and outline perspectives for the search for novel neurotropic drugs to treat various vitamin D-related dysfunctions.

Keywords: Vitamin D, neurosteroid hormone, brain disorders, new drugs.

1. THE VITAMIN D ENDOCRINE SYSTEM

Vitamin D (calciferol) is a fat-soluble seco-steroid hormone synthesized in skin by photolysis of 7-dehydrocholesterol, or ingested with food [27,28,37,39,54,100,101]. The most important biological function of vitamin D is mineral homeostasis, where together with other endocrine hormones it is involved in Ca metabolism by regulating renal and intestinal Ca transport and bone mineralization [58,92,93]. Several additional key functions of vitamin D include the regulation of tissue proliferation, differentiation and apoptosis, as well as cardiovascular (*via* down-regulation of renin-angiotensin system) and immune mechanisms, [84,93,149,150,158].

Vitamin D itself is biologically inert, and its bioactivation involves hydroxylation on carbon 25 in liver (yielding prohormone 25-hydroxyvitamin D, calcidiol, 25-D) followed by subsequent hydroxylation in kidney (Fig. **1**), leading to formation of 1,25-dihydroxyvitamin D (1,25-D, calcitriol) [17,37,41,144,158]. 25-D is the major circulating form of vitamin D [134]. 1,25-D is the main biologically active form of vitamin D [80,81], together with 25-D circulating in blood as complexes with vitamin D-binding protein, albumin, α-fetoprotein, and lipoproteins [30,70]. Activity of kidney 25-hydroxyvitamin D-1α-hydroxylase is down-regulated by 1,25-D, thus tightly controlling the concentrations of 1,25-D in the blood [1,70,112].

In kidneys, both 1,25-D and 25-D are catabolised by oxidation of the side chain by vitamin D-24-hydroxylase (leading to formation of inactive metabolites 24,25-dihydroxyvitamin D and 1,24,25-trihydroxyvitamin D, calcicetrol; Fig. **1**) [109,112,134,135]. This catabolic enzyme is down-regulated by high Ca and P, and up-regulated by 1,25-D, implying its important role in maintaining physiological concentrations of 1,25-D [1,37].

1,25-D regulates the expression of numerous target genes through the nuclear vitamin D receptor (VDR), belonging to a common family of steroid receptors, which also includes steroid, glucocorticoid, and retinoic acid receptors [25,26,33,41,78,100,112,159]. VDR is a 50-60 kDa protein (depending on species), consisting of several functional domains responsible for ligand and DNA binding, heterodimerization, nuclear localization and transcriptional activation [1,74,75,113,158]. The mechanism of the genome effects of vitamin D is similar to that of all other steroid hormones (rev. [94,95]), where DNA-complexed VDR acts as a molecular switch of nuclear 1,25-D signalling to its target genes [25,26]. 1,25-D is the main ligand for VDR, easily penetrating the plasma membrane of its target cells [25,68]. Upon binding 1,25-D, the VDR undergoes a conformational change and forms a complex with a retinoid X receptor (RXR), which bind to DNA elements in the promoter regions of target genes (vitamin D response elements), thus controlling the rate of gene transcription [30,37,68,61]. Approximately 0.5% of the human genome (about 200 genes) are estimated to be primary targets of 1,25-D, although *via* various mechanisms the VDR appears to interfere in the regulation of even more genes [25].

The rapid response to vitamin D uses non-genomic signal transduction pathway, and is believed to occur *via* putative membrane receptors for 1,25-D (VDRm) [14-16,65,112,113]. Although VDRm-like proteins were found in several tissues, including brain [65], their functions and properties are not yet well understood [61]. It is suggested that VDRm is a 60 kD protein with a high affinity to 1,25-D [17,112,113], although it has not yet been cloned, and its domain structure remains unknown (rev. [70]). Fast non-genomic effects of vitamin D occur within seconds, and their signal transduction is thought to involve the formation of second messengers, such as cyclic nucleotides, diacylglycerol, inositol-trisphosphate, and arachidonic acid [14-16,109,128]. Although the area of membrane-dependent vitamin D actions has advances dramatically in the past decades, the evidence supporting this pathway is far more limited, and there are still several key problems that need to be resolved [46,47]. For example, some evidence suggests that the VDR is required for the rapid vitamin D effects [160] and may be identical to VDRm [63], whereas several other candidate proteins have also been suggested to mediate these effects [47,123].

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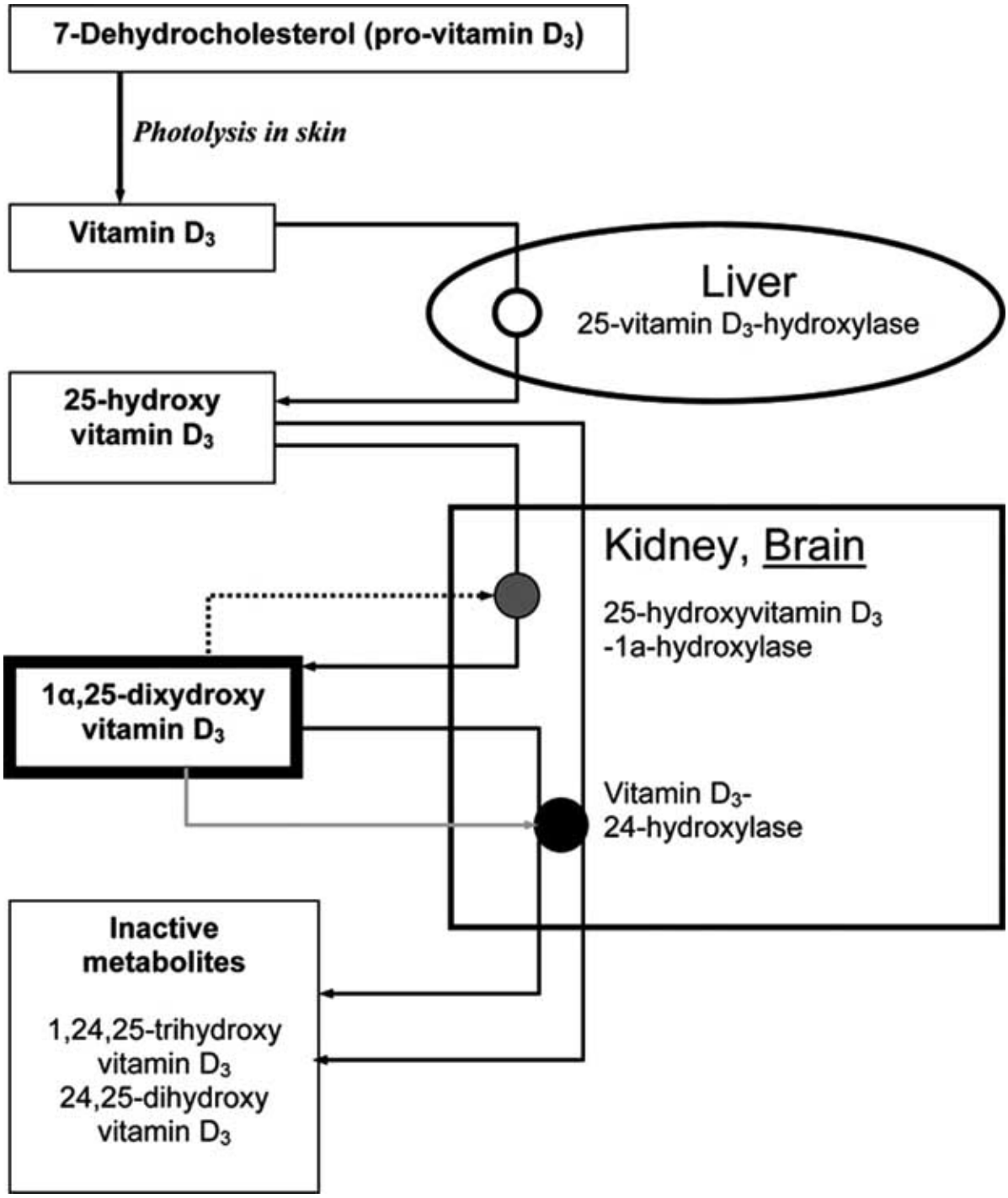

Fig. (1). Synthesis and metabolism of vitamin D. Disrupted line - inhibition, gray line - activation of enzyme activity by vitamin D metabolites.

Clearly, until the rapid non-genomic pathway mechanism and participating proteins are clarified, and their *in vivo* biological roles have been established, targeting this pathway therapeutically will not be justified.

2. THE VITAMIN D AND THE NERVOUS SYSTEMS

Although brain has long been hypothesized to be a target tissue for vitamin D [138-142], the neurobiological role of this hormone was confirmed when the regulatory effects of 1,25-D on neuronal choline acetyltransferase [131] and nerve growth factor [64,110,111,156] were reported. Evidence for the presence of vitamin D, its binding sites, the enzymes of its bioactivation and metabolism (Fig. **1**) and functional VDR in the brain of animals and humans implies that this hormone may serve in the CNS as an autocrine or paracrine neuroactive steroid [28,44,54,108,118,161]. In many brain areas, VDR co-localize with 1α-hydroxylase, the key enzyme of vitamin D bioactivation [44], confirming the link between vitamin D and VDR signaling pathways in the brain. Finally, based on our present knowledge of its physi-

ology, vitamin D meets the criteria for neurosteroid hormones [103,104,124].

Balabanova *et al*. [9] reported that cerebro-spinal fluid concentrations in humans were 8.3 ng/ml for 25-D and 25.0 pg/ml for 1,25-D (approximately 60 pM). 1,25-D crosses the blood-brain barrier and binds to its specific receptors in the brain [70,85]. VDR, actively binding 1,25-D, have been found in brain neurons, glial cells, brain macrophages, spinal cord, and the peripheral nervous system [8,33,55,111,140,153]. In addition, 1,25-D has been reported to activate the expression of VDR in Schwann cells [33]. Likewise, VDRm have also been identified in the brain, where they have been reported to bind 1,25-D [109], suggesting that acting *via* VDR and VDRm, vitamin D may modulate neurotransmitter release and neuronal activity in analogy to other steroid hormones [33,65]. Taken together, these findings imply the important role of this neurosteroid hormone and its receptors in both the central and the peripheral nervous systems [28,53,54,70,75].

Several important functions of vitamin D have to be discussed here, each representing a potentially important target for the creation of novel effective neurotropic drugs. Due to its well-known antiproliferative activity, vitamin D is an important regulator of brain development and differentiation, and its deficiency leads to different brain anomalies, such as longer cortex and lateral ventricles but thinner neocortex and narrower anterior comissure [43-45,86,98]. Interestingly, vitamin D depletion led to more proliferating and fewer apoptotic neurons, but did not affect brain VDR levels, suggesting that the regulation of VDR in the CNS may be more complicated than it was previously recognized [43,86,104]. Behavioural consequences of prenatal vitamin D deficiency in rats include hyperlocomotion, impaired pre-pulse inhibition and altered memory and learning [19-21]. Collectively, these findings suggest that vitamin D-related drugs may be used (at both maternal or neonatal levels) as a preventive therapy, minimizing the risk of developmental and several other related brain disorders, such as schizophrenia [98,104].

Furthermore, anti-proliferation pro-differentiation and pro-apoptotic action of vitamin D leads to its general anti-tumor activity in different tissues [54,161]. Given the expression of VDR in brain tumors, and the ability of 1,25-D to induce apoptosis in such tumor cells [99,162], it is possible to assume the utility of vitamin D (and especially its non-calcemic metabolites) in therapy of brain tumors (Table **1**).

In addition to their developmental and anti-cancer role, vitamin D and VDR are responsible for neuroprotection, occurring *via* multiple mechanisms (see [54,70] for details). Briefly, they include: 1) the reduction of Ca toxicity by stimulation of expression of Ca-binding proteins (calbindins, parvalbumin) or inhibition of expression of L-type Ca channels, 2) the modulation of glutathione metabolism, 3) anti-oxidant-like effects, 4) the reduction of nitric oxide synthesis, 5) the induction of neurotrophins and neuritogenesis, 6) the modulation of cytokine release [13,18,22,23,54,70]. 1,25-D has also been shown to exert robust anti-ischemic effects in the brain cortex, accompanied by significant reduction in heat shock proteins 27 and 32, up-regulation of glial heme oxygenase-1 (metabolizing and detoxifying free heme to endogenous antioxidants biliverdin and bilirubin) and down-regulation of glial fibrillary acidic protein (a sensitive marker for reactive gliosis) [96,97,114]. Therefore, it is possible to assume that novel effective lipophilic neuroprotectors may be created based on vitamin D and its analogs. Finally, accompanied by well-known anti-hypertensive properties of this hormone [92,93,161], the use of such drugs may also be a rational strategy for neuroprotection in high-risk subjects, such as hypertensive patients (Table **1**).

Special attention has to be given to pro- and anti-apoptotic mechanisms mediated by vitamin D and its analogs in the brain at different stages of ontogenesis. For example, prenatal vitamin D depletion in rats down-regulates pro-apoptotic genes, whereas clear reversal of this trend was seen postnatally, suggesting that vitamin D is crucial for normal sequence of apoptotic and mitotic activity during brain development [86]. In adult brain, vitamin D generally up-regulates pro-apoptotic and down-regulates anti-apoptotic mechanisms [132], thus, contributing to overall neuroprotective effects of this hormone.

Pronounced immuno-modulating properties of vitamin D [13,24] imply its potential role in the neuro-immune interactions. Indeed, low vitamin D status has been implicated in the etiology of autoimmune diseases such as multiple sclerosis (MS), rheumatoid arthritis, insulin-dependent diabetes mellitus, and inflammatory bowel disease [22,31,37,105]. The prevalence of MS is highest where environmental supplies of vitamin D are lowest, strengthening the potential role of this hormone as a natural inhibitor of MS [59,107,151].

Table 1. Summary of Potential Neurotropic Drugs Acting *via* the Vitamin D/VDR Neuroendocrine System

Potential drugs	Possible mechanisms of action and evidence	Key references
Developmental	Preventive therapy, minimizing the risk factors of developmental and other related brain disorders	[43,44,98, 103,104]
Anti-cancer	General anti-cancer anti-proliferation, pro-differentiating effects of vitamin D and metabolites	[40,53,99, 161,162]
Antiepileptics	Chronic vitamin D supplementation Direct fast anti-epileptic effects of 1,25-D	[4,6,32] [77,129]
Neuroprotectors	Reduction of Ca toxicity, increased antioxidant protection, modulation of cytokine network	[28,54,70]
Neuro-immunomodulators	VDR-mediated immuno-suppressant effects	[22-24]
Antidepressants	Antidepressant-like effects of vitamin D and/or sun therapy. Mechanisms of action are yet unknown.	[56,141]
Anxyolitics (?)	This possibility is based on mutant mouse data. Potential mechanisms of action are unknown.	[71,74,75]
Combined action	E.g., cardiotropic + neuroprotective, antiepileptic + neuroprotective.	See text for details

The systemic and local increase in the expression of several anti-inflammatory cytokines by 1,25-D is responsible for the ability of vitamin D to block, in a Ca-dependent manner, experimental encephalomyelitis, an animal model of MS [22-24]. Interestingly, the hormone precursor vitamin D_3 itself inhibited this pathology only in female mice [133], suggesting gender-specific differences in metabolism of this hormone in the CNS. Anti-MS effects of vitamin D may involve paracrine or autocrine metabolism of 25-D by target cells, altered macrophage, dendritic and T-cells functions, their sensitization to apoptotic signals, as well as some action on the CNS component of MS pathogenesis [132,151], and generally seem to be associated with genomic VDR-mediated pathways [22,105]. Therefore, vitamin D and its analogs represent a base for creation of novel anti-MS drugs, targeting the vitamin D neuroendocrine system (Table **1**).

A growing body of literature suggests the link between vitamin D-related disorders and epilepsy [4,6,28,57,62]. In humans, seizures accompanied by hypocalcemia and lowered vitamin D levels are often seen in patients with hereditary or nutritional rickets, whereas vitamin D and Ca therapy have long been known to reduce seizures [4,32,67,60,107, 115,116]. At the same time, chronic treatment with antiepileptic drugs impairs mineral homeostasis in epileptic patients, leading to a marked hypocalcemia and reduced plasma levels of vitamin D (which in turn may increase seizure) [4,62]. These observations have led to a wide practice of using vitamin D in epilepsy, as both the main and supplementary therapy, especially in cases complicated by side-effects of chronic antiepileptic drugs [4,32,76].

However, there are mounting evidence indicating that vitamin D *per se* may play a significant role in epilepsy. In their pioneering study, Siegel *et al*. [129] reported direct anticonvulsant effects (increased seizure threshold in rats following the electrical stimulation of the dorsal hippocampus) within 5-10 min after i.c.v. or i.v. injection of 1,25-D, indicating the potential role of vitamin D in epilepsy, and the possibility of direct anticonvulsant properties of this neurosteroid hormone. Consistent with this, our recent study showed acute anticonvulsant effects of 1,25-D in the model of chemically-induced seizures in mice [76]. 1,25-D affected predominantly the more severe stages of seizures, showing the anticonvulsant profile which is clinically relevant and may be of interest for potential application [76]. The ability of 1,25-D to reduce seizures occurred within a relatively short (40 min) time following s.c. administration, suggesting that its genomic effects may not be involved in its antiepileptic profile reported in these two studies [76,129], and raising the possibility that "fast" anticonvulsant properties of 1,25-D may represent a potential rationale for novel antiepileptic drugs.

Furthermore, numerous data link the vitamin D system to the regulation of behaviour [5,12]. VDR are found in key brain areas including the cortex, cerebellum and limbic system, all known to regulate behaviour [87,120,155]. In humans, vitamin D deficiency has long been known to be accompanied by irritability, depression, psychoses and defects in mental development [28], whereas 25-D and 1,25-D levels were significantly lower in patients suffering from depression, alcoholism and psychoses [126]. The psychotropic mood-elevating effects of vitamin D have also been well-documented in the literature [55,88,141]. In animals, vitamin D deficiency produces behavioural alterations including decreased exploration and maze performance [5], while neonatal treatment with vitamin D has been shown to affect sexual behaviours in rats [106]. Prufer and Jirikowski (1997) reported neuronal co-localization of VDR with oxytocin, suggesting a direct genomic action of this steroid on oxytocin expression, similar to other effects of steroid hormones on peptidergetic systems [119]. Collectively, this suggests that vitamin D could be an important factor controlling key brain functions and behaviours in both animals and humans [19-20,28,71-75].

3. DATA FROM MUTAGENESIS

Human VDR genes are highly polymorphic, and their variations (missence and nonsence mutations, splice site mutations, and partial deletion) occur frequently in the population, dramatically affecting DNA-binding, nuclear localization, ligand-binding, heterodimerization and other functions of the VDR [100,101]. These mutations cause various vitamin D-related dysfunctions (leading to partial or total hormone resistance and rickets), and demonstrate the variability in the vitamin D/VDR endocrine system [100,101,149,150]. The fact that they may also lead to several psychiatric phenotypes [117,145,157], further confirms the important role of the vitamin D/VDR neuroendocrine functions in the regulation of behaviour.

Mouse mutagenesis data further support this notion. Mice with genetically impaired VDR (knockout mice, KO) are currently available for biomedical research focusing on the biological functions of vitamin D and VDR [69,130]. Several groups have generated VDR KO mice by targeted disruption of different fragments of this gene [42,89,152,159]. All these mice display similar physiological phenotypes, with pronounced rickets, resembling human hereditary vitamin D-resistant rickets type II [89,159]. VDR KO mice demonstrate alopecia, hypocalcemia, hypophosphatemia, elevated plasma vitamin D (due to the lack of VDR-mediated negative feedback), impaired reproductive system, muscular and skeletal abnormalities, and normally die by 15 weeks [89,159]. Although rescue high Ca/P diet has been shown to completely prevent most of these physiological anomalies, several aberrant features were not corrected in these mice, including alopecia and reduced brain levels of calbindin D9k, implying direct VDR-dependent Ca/P-independent mechanisms [35,66,90,91]. Since the absence of functional VDR results in the target tissue insensitivity to genomic effects vitamin D, the analysis of the behaviour of these mutant mice seems to be an important tool to assess the role of the vitamin D/VDR system in the brain.

Behavioural phenotyping of various mutant mice is an important part of neuroscience research, allowing us to establish the link between genes and brain disorders [34,71,74]. Ca/P-rich diet has been shown to dramatically extend the lifespan of VDR KO, enabling the establishment of their behavioural phenotypes [21,71-75]. The unique physiology of these mice (the lack of VDR in combination with elevated plasma 1,25-D) makes them particularly suitable to dissect different mechanisms of vitamin D action in the regulation of behaviour [75]. For example, unimpaired behavioural phenotypes of these mutants would imply VDR-

independent mechanisms, whereas altered behaviours would support the important role of VDR and VDR-mediated mechanisms in the regulation of behaviour [77].

Overall, marked behavioural differences were found in a battery of tests, showing high anxiety and aberrant grooming phenotype in mice lacking VDR [71-75]. In line with this, the highest brain VDR concentration has been found in the limbic system, the key emotiogenic brain structure, and its extensions in the brain, also involved in the regulation of grooming [155]. Notably, male mice fed with special rescue Ca/P-rich diet, showed no overt anomalies in their sexual behaviours, clearly indicating that sexual behaviour in male VDR KO mice is unaffected by the VDR genetic ablation [74]. VDR KO mice also displayed specific motor impairments (poor swimming and vertical screen retention), indicating serious motor defects in tests requiring rigorous activity [72].

Analysis of maternal and nest-building activity is an important part of behavioural phenotyping, and their abnormalities may indicate serious brain dysfunctions [34]. Overall, male and female KO mice built less complex and incomplete nests, indicating that VDR genetic ablation impairs nest-building behavioural domain [75]. Although VDR KO females mice have long been known to be infertile [159], a high Ca diet has been recently shown to restore their fertility [66]. Nevertheless, we observed a dramatic impairment of the VDR KO maternal behaviour, manifest in poor nest building (predominantly cup-shaped), abnormal mothering styles, and 100% cannibalism [75]. Notably, numerous data show the important role of VDR in the regulation of prolactin gene expression in different tissues [29,38], suggesting that the vitamin D/VDR and prolactin endocrine systems may interact [122]. Thus, it was possible to assume that such interaction is impaired in VDR KO mice, leading to their abnormal prolactin-dependent mechanisms. Given the key role of prolactin in the regulation of nest-building and maternal behaviours in mice [154], this hypothesis, if true, may explain both abnormal maternal and nest-building behaviours, observed in the VDR KO mice [75]. Moreover, co-localization of VDR with oxytocin in hypothalamic neurons [119] implies some degree of interplay between the oxytocin and vitamin D endocrine systems. Given the key role of oxytocin in maternal behaviour [121], and possible impairment of oxytocin-VDR interplay in mice lacking VDR, this may also contribute to disturbed maternal behaviours observed in VDR KO mice.

Recently, some additional anomalies have been reported for these mice, including aberrant behaviours and seizure sensitivity [21,77], emphasizing the growing interest in the use of the VDR genetic ablation as an animal model of human vitamin D-related brain disorders. From this point of view, other mutant mice with abnormalities in the vitamin D system (e.g., mice lacking 25-dihydroxyvitamin D-1α-hydroxylase [82,83,134] or 25-hydroxyvitamin D-24-hydroxylase [135,136]) may also be a useful tool for such studies.

4. NEW DIRECTIONS OF RESEARCH AND CONCLUDING REMARKS

Overall, several potential mechanisms may underlie neurotropic activity of 1,25-D. For example, it is possible to assume that this steroid hormone modulates the brain neuromediators and receptors. There are data showing that metabolism of acetylcholine and dopamine are regulated by 1,25-D (see [28,54,75] for details). In addition, since gamma amino butyric GABA-A receptors represent an important target for non-genomic action of many neurosteroids and neuroactive hormones [94,95], it is tempting to speculate that 1,25-D may act in the brain in a similar way, modulating neuronal excitability and other neurophysiological phenomena. Given the crucial role of GABAergic system in brain pathogenesis (such as anxiety and epilepsy), the possibility of steroid-like "fast" effects of 1,25-D on GABA-A receptors seems indeed likely. Moreover, vitamin D may also modulate the GABAergic system at a genomic level. For example, altered vitamin D levels have been recently reported to affect the expression of α4 and α1 subunits of GABA-A receptors [45]. Given the key role of GABAergic system in the regulation of brain functions [77], these aspects of vitamin D-GABA interaction justify further in-depth studies, and may lead to a potentially interesting therapy strategy based on genomic modulation of central GABAeric system.

Moreover, it is possible to expect that vitamin D interplays with other steroid and similar hormones acting in the brain. For example, early findings indicated that VDR in the pituitary may be regulated by thyroid hormone (TH), and that 1,25-D may affect the anterior pituitary [79]. In line with this, TH receptor-associated protein TRAP220 interacts with both TH receptors and VDR and is found in the brain [52], where it may play an important role by triggering the effects of the two steroids on brain development. Likewise, TH might affect the signal transduction of retinoid, vitamin D and TH by changing RXR levels in different tissues, including the brain [102]. Such interplay becomes even more important, given the role of TH in the regulation of behaviour [52]. Thus, drugs synergetically targeting vitamin D and other steroid hormones, or modulating steroid-vitamin D interplay, may lead to a new class of CNS drugs.

Importantly, neuroendocrine interactions with vitamin D are not limited only to steroids, and may also involve peptidergic mechanisms. As already mentioned, vitamin D seems to interact with prolactinergic mechanisms in different organs, including the CNS [147,148]. On the other hand, several studies showed that prolactin may be involved in the regulation of Ca^{++} homeostasis [10,48,49,122], suggesting possible evolutionary nature of such interplay between the two Ca-regulating hormones. Recently, prolactin has been reported to influence Ca^{++} levels affected by acute stressors [50,51]. Therefore, the search for novel neurotpopic drugs targeting both the vitamin D and prolactinergic systems may represent a new direction of CNS drugs research, especially in the field of anti-stress Ca-normalizing agents.

In line with this, 1,25-D has been reported to modulate pituitary thyrotropin secretion [36,125] and up-regulate the expression of thyrotropin releasing hormone (TRH) receptors [7]. Thus, given their important role in the brain, pharmacological modulation of vitamin D interplay with these hormones may represent another potential target in CNS drug research.

Since vitamin D plays an important role in the regulation of Ca^{++} homeostasis, another possibility for its brain action can be altered Ca^{++} metabolism. For example, 1,25-D has

rapid effects on Ca^{++} absorption from the intestine and other organs, and may lead to increased plasma and reduced brain Ca^{++} concentrations, thus contributing to overall reduction of neuronal excitation. However, our study [76] dissociated calcemic effects of this hormone from its antiepileptic action. In line with this, vitamin D treatment with 4000-16000 IU/day led to robust clinical antiepileptic effects not related to altered plasma Ca^{++} levels (see [4] for review). Importantly, the Ca level is not the only determining factor for the occurrence of seizures [4]. Indeed, while some seizures initially do not respond to Ca^{++} therapy but are easily corrected with vitamin D, individual thresholds may also be an important factor for seizure pathogenesis [4,129]. It is therefore possible to suggest that vitamin D, perhaps acting in a neurosteroid-like manner, may be involved in "fine tuning" of neuronal excitability at the threshold level. In line with this hypothesis, lower circulating vitamin D levels are reported to increase antiepileptic efficiency of 1,25-D [129], suggesting possible modulation of brain activity by vitamin D.

Collectively, this outlines the importance of the search for novel antiepileptic drugs based on selective non-toxic vitamin D-related ligands (e.g., [146]). For example, finding a steroid vitamin D-related compound with both vitamin D-like and GABA-modulating properties, if successful, could lead to a highly effective therapy targeting several parallel pathogenic mechanisms of epilepsy. Moreover, since the link between neuroprotective and antiepileptic mechanisms has long been accepted, the use of vitamin D as a complex "combined" therapy with both neuroprotective and antiepileptic profiles may also be of clinical importance (Table **1**). In general, such properties of vitamin D raise the possibility of synergism in its therapeutical properties, allowing to combine several different mechanisms of action to treat various brain disorders. In addition, possible action of vitamin D on glial cells may also be important, especially given their crucial role in glutamate metabolism, providing glutamine as a precursor for both GABA, glutamate as well as for glutathione. Therefore, both neuronal and glial vitamin D-mediated mechanisms may be involved in antiepileptic and other neurotropic effects of vitamin D [53].

Interestingly, co-localization of VDR with calbindins in the brain suggests their potential interaction [143]. In VDR KO mice, calbindin-D9k (but not calbindin-D28k) mRNA was dramatically reduced in the brain, strengthening the link between VDR and calcium-binding proteins in the CNS [91]. Since Ca-binding proteins are important for brain functions, and their imbalance results in marked behavioural and sensory deficits in mice [2,11,127], it is possible that vitamin D modulates the expression of brain endogenous Ca-binding proteins, thus influencing a variety of brain processes. In line with this, 1,25-D has been recently shown to up-regulate calbindin-D28k and parvalbumin in motoneuron cells [3], further supporting the role of vitamin D/calcium binding proteins interplay in the regulation of the motor system and behaviour.

Finally, although the mechanisms of antidepressant-like effects of vitamin D have not yet been established [56], the ability to influence several neuromediators and key brain proteins (see above) may be responsible for such biological activity of this neurosteroid, most likely mediated *via* VDR. Consistent with this notion, bipolar and unipolar disorders were increased in patients with VDR mutation [117], further confirming the link between the vitamin D/VDR system and depression [141].

In conclusion, we shall note that the major problem with vitamin D therapy (especially using 1,25-D) is its toxic effects due to hypercalcemia [28]. Therefore, creation of novel drugs targeting the vitamin D neuroendocrine system shall focus on its low-calcemic analogs, retaining other beneficial therapeutical properties of vitamin D [137]. Furthermore, based on these studies, it is possible to expect that the development of synthetic vitamin D ligands with tissue-specific uptake (e.g., [85]) may lead to a new class of highly selective vitamin D-related drugs specifically targeting the brain. In general, the data summarized here (Tables **1** and **2**) give further support to the crucial role of vitamin D in the brain, and contribute to the growing recognition of the importance of this neurosteroid hormone as a potential target for various classes of CNS drugs.

Table 2. Summary of Possible Mechanisms of Action of Vitamin D-Related Ligands in the Brain

I. VDR-related mechanisms (neuronal, glial cells)
• Brain development and differentiation
• Apoptosis
• Neuroprotection
• Neurotrophins and cytokine release
• Expression of neurotransmitter metabolism enzymes
• Expression of neuronal receptors (GABA, etc. ?)
• Interplay with other steroid hormones
• Modulation of peptidergic systems
• Expression of Ca channels and Ca-binding proteins
II. VDR-unrelated mechanisms
• VDRm-directed action?
• Potential direct steroid-like effects
- Membranotropic effects?
- Neurosteroid-like modulation of neurotransmitter receptors?
III. Secondary (indirect) mechanisms
• Altered Ca/P metabolism
• Renin-angiotensin system, hypertension
• Other?

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ABBREVIATIONS

CNS = Central nervous system

DNA = Deoxyribonucleic acid

GABA = Gamma aminobuturic acid

KO = Knockout mice

MS = Multiple sclerosis

RXR = Retinoic acid X receptor

TH = Thyroid hormone

TRH = Thyrotropin releasing hormone

VDR = Nuclear vitamin D receptor

VDRm = Membrane vitamin D receptor

1,25-D = 1,25-Dihydroxyvitamin D

25-D = 25-Hydroxyvitamin D

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