# New clues about vitamin D functions in the nervous system

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**Accumulating data have provided evidence that 1α,25 dihydroxyvitamin $D_3$ [1,25-$(OH)_2D_3$] is involved in brain function. Thus, the nuclear receptor for 1,25-$(OH)_2D_3$ has been localized in neurons and glial cells. Genes encoding the enzymes involved in the metabolism of this hormone are also expressed in brain cells. The reported biological effects of 1,25-$(OH)_2D_3$ in the nervous system include the biosynthesis of neurotrophic factors and at least one enzyme involved in neurotransmitter synthesis. 1,25-$(OH)_2D_3$ can also inhibit the synthesis of inducible nitric oxide synthase and increase glutathione levels, suggesting a role for the hormone in brain detoxification pathways. Neuroprotective and immunomodulatory effects of this hormone have been described in several experimental models, indicating the potential value of 1,25-$(OH)_2D_3$ pharmacological analogs in neurodegenerative and neuroimmune diseases. In addition, 1,25-$(OH)_2D_3$ induces glioma cell death, making the hormone of potential interest in the management of brain tumors. These results reveal previously unsuspected roles for 1,25-$(OH)_2D_3$ in brain function and suggest possible areas of future research.**

For several decades, the role of vitamin D was thought to be confined to $Ca^{2+}$ and phosphate homeostasis, and to bone formation and maintenance. However, current evidence suggests a wider biological role for 1α,25 dihydroxyvitamin $D_3$ [1,25-$(OH)_2D_3$] in tissues not primarily related to mineral metabolism. This review focuses on the recent advances in our understanding of the biology of 1,25-$(OH)_2D_3$ in the nervous system and aims to be a source of information and inspiration for the development of new ideas and hypotheses on the possible functions of 1,25-$(OH)_2D_3$ in the brain.

## Brain 1,25-$(OH)_2D_3$ synthesis and degradation

Vitamin $D_3$ can either be ingested, or synthesized in the skin from 7-dehydrocholesterol during exposure to sunlight in a reaction that is catalyzed by ultraviolet B radiation [1]. In both cases, vitamin $D_3$ is biologically inert and requires a two-step enzymatic activation (Fig. 1). It was assumed that the brain 1,25-$(OH)_2D_3$ supply was dependent on the plasma concentration of 1,25-$(OH)_2D_3$ [2–4]. However, recent data demonstrating the brain localization of vitamin $D_3$ 25-hydroxylase and 25-hydroxyvitamin $D_3$-1α-hydroxylase enzymes have challenged this conclusion and suggest that the central nervous system (CNS) can locally perform the bioactivation of the vitamin $D_3$ prohormone [5–7]. In this regard, it is noteworthy that microglial cells in culture produce 1,25-$(OH)_2D_3$ from its precursor [8]. The localization of the enzymes involved in the biosynthesis of 1,25-$(OH)_2D_3$ in the brain and the presence of 1,25-$(OH)_2D_3$ in the cerebrospinal fluid [3] suggest the existence of a catabolic pathway for this hormone in the CNS. The first step of the major pathway involved in the inactivation of this hormone is achieved by vitamin $D_3$ 24-hydroxylase and, as expected, the corresponding gene is upregulated in glial cells exposed to 1,25-$(OH)_2D_3$ [9]. Thus, together, these results suggest the existence of both the biosynthetic and the degradative pathways for 1,25-$(OH)_2D_3$ in the brain.

## Molecular mechanism of action of 1,25-$(OH)_2D_3$

### *The vitamin D receptor (VDR)*

The nuclear functions of 1,25-$(OH)_2D_3$ are mediated through the vitamin D receptor (VDR) [10,11]. Since the initial reports of Stumpf and collaborators on the presence of vitamin D-specific nuclear binding in brain and spinal cord [12–14], evidence has accumulated to suggest that both mRNA encoding the VDR and the protein itself are present in the nervous system. Thus, *VDR* gene expression has been demonstrated in neuronal and glial cells [15–23]. VDR belongs to the nuclear hormone receptor superfamily and acts as a ligand-inducible transcription factor. It regulates gene expression by binding to specific DNA-response elements of target genes after heterodimerization with the retinoid X receptor and the recruitment of several nuclear receptor coactivator proteins, including members of the steroid receptor coactivator family [24–26]. Heterodimerizations of the VDR with the nuclear receptors for all-*trans* retinoic acid and thyroid hormone or with Smad3 [a member of the SMAD protein family transducing the signal for transforming growth factor β (TGF-β)] have also been reported, and suggest the existence of crosstalk between these signaling pathways [27,28]. These heterodimerizations could play a role in neuro-ontogenesis because *VDR* expression is regulated developmentally within nervous tissues [19,29]. In keeping with this notion is the fact that VDR is found in the neuroepithelium during early neurogenesis and, at later stages, in one of the major CNS areas able to maintain neural stem cell generation throughout life: the subventricular zone [19]. Moreover, the *VDR* gene is specifically expressed within developing neurons of rodent dorsal root ganglia, suggesting a role for vitamin D in peripheral nervous system development [18].

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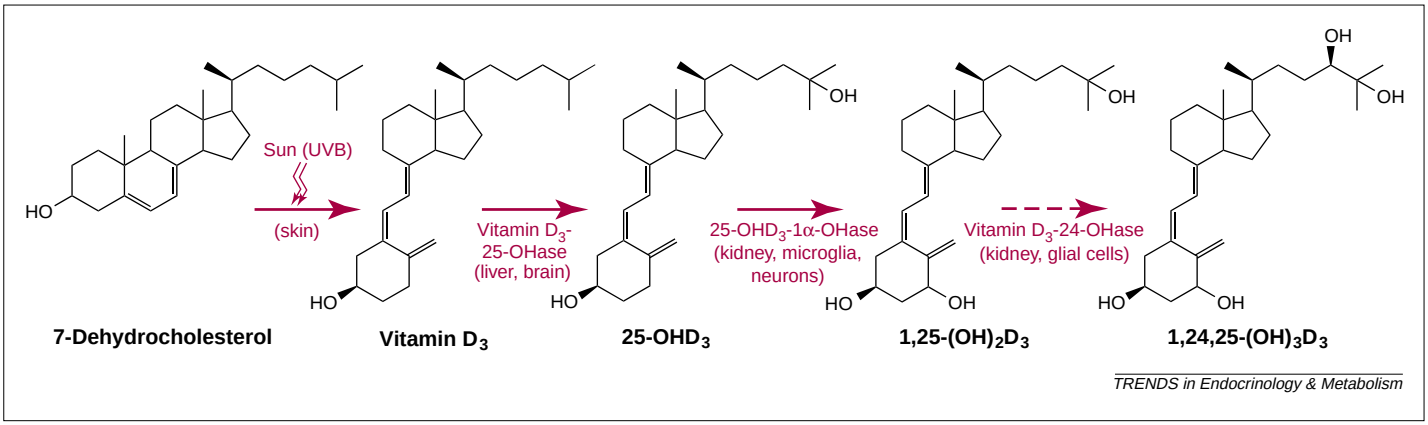


Fig. 1. Vitamin D metabolism. Vitamin D derived from the diet or from the ultraviolet B (UVB) light-induced conversion of skin 7-dehydrocholesterol is first 25-hydroxylated in the liver and subsequently 1α-hydroxylated in the kidney to give 1,25-$(OH)_2D_3$. The two enzymes involved in this bioactivation process are the vitamin $D_3$ 25-hydroxylase (vitamin $D_3$ 25-OHase) and the 25-hydroxyvitamin $D_3$-1α-hydroxylase (25-$OHD_3$-1α-OHase). The presence in brain cells of the enzymes involved in this activation process suggests that 1,25-$(OH)_2D_3$ metabolism also occurs in the brain. Indeed, brain microglial cells have already been reported to synthesize 1,25-$(OH)_2D_3$ *in vitro*. The bold arrows indicate the activation sequence and the dashed arrow the first step of the inactivation sequence.

*Non-genomic actions*

Non-genomic effects have also been described for steroids and thyroid hormones. These effects are probably mediated via specific membrane receptors [30,31]. Hence, the rapid, non-genomic effects of 1,25-$(OH)_2D_3$ could be mediated by interaction of the hormone with a specific plasma membrane receptor. Regulation of $Ca^{2+}$ channels and activation of protein kinase C and mitogen-activated protein kinase pathways are some of the non-genomic signal transduction events triggered by 1,25-$(OH)_2D_3$ [32]. A putative plasmalemmal receptor for 1,25-$(OH)_2D_3$ has recently been detected in chick brain [33]. It is tempting to speculate that 1,25-$(OH)_2D_3$ could act in the brain in a similar way to neuroactive steroids by modulating neuron excitability and other electrophysiological phenomena [34,35].

## 1,25-$(OH)_2D_3$ and the nervous system

*Neuroprotective effects of 1,25-(OH)2D3*

Since the regulation of nerve growth factor (NGF) synthesis by 1,25-$(OH)_2D_3$ was first reported [36], various studies have demonstrated that 1,25-$(OH)_2D_3$ can act on cells of the nervous system by modulating the production of neurotrophins (Fig. 2; Table 1). For instance, the synthesis of NGF [17,20,37], neurotrophin 3 (NT3) [38] and glial cell line-derived neurotrophic factor (GDNF) [39] was upregulated by 1,25-$(OH)_2D_3$, whereas neurotrophin 4 (NT4) was downregulated [38]. In several cases, stimulation of neurotrophin production by 1,25-$(OH)_2D_3$ was correlated with a neuroprotective effect [40,41]. In this regard, it is noteworthy that 1,25-$(OH)_2D_3$ has recently been shown to attenuate the hypokinesia and neurotoxicity induced by 6-hydroxydopamine in rats [42]. These data indicate that 1,25-$(OH)_2D_3$, or its synthetic analogs, could be valuable in the treatment of neurodegenerative diseases. In addition to its influence on neurotrophin synthesis, 1,25-$(OH)_2D_3$ could mediate its neuroprotective effects via the modulation of neuronal $Ca^{2+}$ homeostasis. In support of this is the recent report of downregulation of the L-type voltage-sensitive $Ca^{2+}$ channel in hippocampal neurons in the presence of 1,25-$(OH)_2D_3$, which has been correlated with a neuroprotective effect against excitotoxic insults [43]. Another way in which 1,25-$(OH)_2D_3$ might mediate its neuroprotective effect is to induce the synthesis of $Ca^{2+}$-binding proteins, such as parvalbumin [44]. 1,25-$(OH)_2D_3$ has also been reported to inhibit the synthesis of inducible nitric oxide synthase (iNOS) [45,46], an enzyme induced in CNS neurons and non-neuronal cells during various insults or diseases, such as ischemia, Alzheimer's disease, Parkinson's disease, AIDS, infections, multiple sclerosis and experimental autoimmune encephalomyelitis (EAE). iNOS produces nitric oxide, one of the biological effects of which is to damage both neurons and oligodendrocytes when it is produced at high levels [47,48]. Similarly, a partial reduction in lipopolysaccharide-induced levels of mRNA encoding macrophage colony-stimulating factor (M-CSF) and tumor necrosis factor α (TNF-α) is seen in 1,25-$(OH)_2D_3$-treated astrocytes [49]. 1,25-$(OH)_2D_3$ has also been reported to upregulate γ-glutamyl transpeptidase activity and expression of the corresponding gene in rat brain [50,51]. Because γ-glutamyl transpeptidase is largely involved in the glutathione cycle of the brain in crosstalk between astrocytes and neurons [52,53], it has been proposed that 1,25-$(OH)_2D_3$ could control brain detoxification processes [50,51]. An increase in glutathione levels, linked to a neuroprotective effect of 1,25-$(OH)_2D_3$, has been reported in mesencephalic dopaminergic neurons treated with the inhibitor of glutathione synthesis, L-buthionine sulfoximine, and with the neurotoxin 1-methyl-4-phenylpyridine [53].

*Neurotransmitter biosynthesis*

The finding that 1,25-$(OH)_2D_3$ treatment results in an increase in choline acetyltransferase activity in specific rat brain nuclei has prompted the suggestion that this hormone might influence certain aspects of anterior pituitary lobe function [54]. Another study has reported that vitamin D deficiency in the

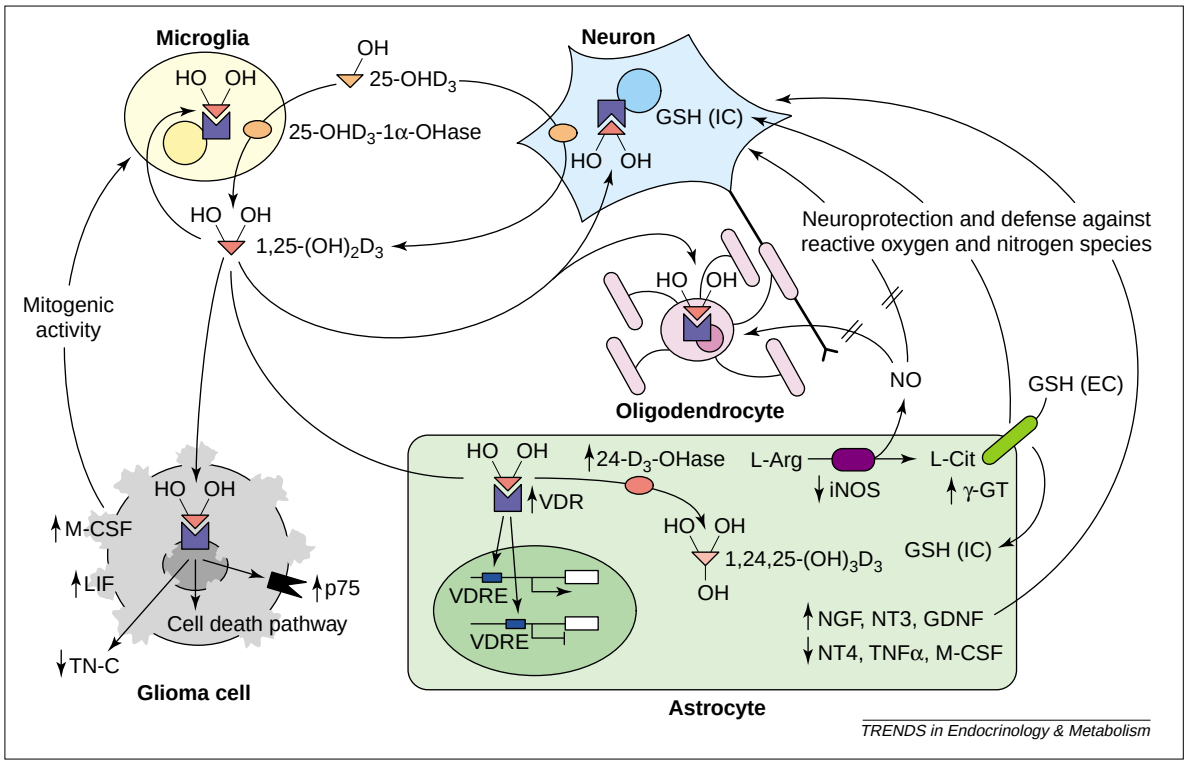


Fig. 2. Proposed overview of the possible brain vitamin D signaling pathway. Local synthesis of 1,25-$(OH)_2D_3$ might be performed by 25-$OHD_3$-1α-OHase-expressing cells, such as neurons, in addition to activated microglia. This local synthesis of 1,25-$(OH)_2D_3$ by microglia could represent a central nervous system anti-tumor response, because 1,25-$(OH)_2D_3$ induces cell death and/or redifferentiation programs in glioma cells. The 1,25-$(OH)_2D_3$ present in the brain can be further metabolized by 24-$D_3$-OHase-expressing cells, such as astrocytes. Glial and neuronal cells that express the *VDR* gene are target cells for 1,25-$(OH)_2D_3$. In astrocytes, 1,25-$(OH)_2D_3$ upregulates the synthesis of several neurotrophins, including NGF, NT3 and GDNF, and of γ-glutamyl transpeptidase, which could be involved in the neuroprotective effects of 1,25-$(OH)_2D_3$. Inhibition of the synthesis of iNOS by 1,25-$(OH)_2D_3$ is another way to mediate 1,25-$(OH)_2D_3$ neuroprotective effects. Large amounts of NO are toxic for both neurons and oligodendrocytes (the CNS-myelinating cells). NO can rapidly react with oxygen species to form highly harmful products such as hydroxyl and nitrogen dioxide radicals. Intracellular GSH, provided from extracellular GSH by serial enzymatic steps involving astrocytic γ-GT, could then be used to prevent the formation of reactive nitrogen or oxygen radicals in these cells. Abbreviations: 1,25-$(OH)_2D_3$, 1α, 25-dihydroxyvitamin $D_3$; 1,24,25-$(OH)_3D_3$, 1α,24,25-trihydroxyvitamin $D_3$; 24-$D_3$-OHase, vitamin $D_3$-24-hydroxylase; 25-(OH)-$D_3$-1α-OHase, 25-hydroxyvitamin $D_3$-1α-hydroxylase; 25-$OHD_3$-1α-OHase, 25-hydroxyvitamin $D_3$-1α-hydroxylase; EC, extracellular; γ-GT, γ-glutamyl transpeptidase; GDNF, glial cell line-derived neurotrophic factor; GSH, glutathione; IC, intracellular; iNOS, inducible nitric oxide synthase; L-Arg, L-Arginine; L-Cit, L-Citrulline; LIF, leukemia inhibitory factor; M-CSF, macrophage colony-stimulating factor; NGF, nerve growth factor; NO, nitric oxide; p75, low-affinity NGF receptor; TN-C, tenascin C; TNF-α, tumor necrosis factor α; NT3, neurotrophin 3; NT4, neurotrophin 4; VDR, vitamin D receptor; VDRE, vitamin D-responsive element.

weanling rat increased the dopamine concentration in the cortex [55]. 1,25-$(OH)_2D_3$ also increases expression of the gene encoding tyrosine hydroxylase in adrenal chromaffin cells [56]. The extension of this regulation to neurons could be of interest, because tyrosine hydroxylase is the rate-limiting enzyme in the biosynthesis of catecholamines.

## 1,25-$(OH)_2D_3$ and nervous system diseases

### *CNS tumors*

The potential role of vitamin D in the treatment of cancer was first recognized in 1981 in myeloid leukemic cells [57]. It was then extended to several other malignancies, including breast, prostate and colon carcinoma. These antiproliferative properties have led to the development of numerous pharmacological analogs that inhibit cancer cell growth with reduced calcemic activity. In CNS tumors, 1,25-$(OH)_2D_3$ and several synthetic analogs are effective in inducing a cell death pathway in glioma cells [58–60]. This effect requires *VDR* gene expression [61] and could involve the upregulation of the p75 receptor by 1,25-$(OH)_2D_3$ [62,63]. In parallel, 1,25-$(OH)_2D_3$ inhibits, in rat C6 glioma cells, the synthesis of tenascin-C, an extracellular matrix protein that displays growth-promoting, invasive and pro-angiogenic properties [64], and increases the expression of the genes encoding M-CSF and leukemia inhibitory factor (LIF) [49]. This suggests alternative mechanisms for the anticancer activity of 1,25-$(OH)_2D_3$ within the brain. More recently, the 1,25-$(OH)_2D_3$ sensitivity of glioma cells has been extended to several glioma cell lines and primary cultures derived from surgical specimens [65]. Furthermore, beneficial effects of vitamin D therapy in the treatment of glioblastomas have been reported in a phase II clinical study [66]. In addition to their potential interest in the treatment of glioma, these results raise the possibility that the local synthesis of 1,25-$(OH)_2D_3$ by microglial cells in the vicinity of tumor cells could be one of the mechanisms involved in the control of glioma cell proliferation [8].

**Table 1. Molecular and cellular effects of 1,25-$(OH)_2D_3$ on neural tissues[a,b]**

| Physiological relevance | 1,25-$(OH)_2D_3$ effects | Target cells and tissues | Refs |
|---|---|---|---|
| Vitamin D metabolism and signaling | 24-OHase (mRNA) ↗ | Astrocytes | [9] |
| | VDR (mRNA) ↗ | Astrocytes, glioma cells, schwann cells, oligodendrocytes | [17,20,22] |
| Growth factors metabolism and signaling | NGF (mRNA/protein) ↗ | Adult rat brain, astrocytes, schwann cells | [17,20,34,37,41] |
| | NT-3 (mRNA) ↗ | Astrocytes | [38] |
| | NT-4 (mRNA) ↘ | Astrocytes | [38] |
| | GDNF (mRNA) ↗ | Glioma cells | [39] |
| Neurotransmission and neurotransmitters metabolism and signaling | L-VSCC (mRNA/protein) ↘ | Hippocampal neurons | [43] |
| | TH (mRNA) ↗ | Adrenal chromaffin cells | [56] |
| | ChAT activity ↗ | Adult rat median eminence and bed nucleus of the stria terminalis | [54] |
| Neuroprotection | Neurotoxicity associated with ischemia ↘ | Adult rat cortex | [40] |
| | Neurotoxicity associated to experimental diabetic neuropathy ↘ | Adult rat peripheral nervous system | [41] |
| | 6-OHDA toxicity ↘ | Adult rat brain | [42] |
| | Parvalbumin levels ↗ | Adult rat striatum | [44] |
| | γ-GT (mRNA/specific activity) ↗ | Adult rat brain, astrocytes, pericytes | [50,51] |
| | GSH levels ↗ | Astrocytes, dopaminergic neurons | [51,53] |
| Neuroimmune interactions | EAE physiopathology ↘ | Adult rat brain, adult mice brain | [45,68–70] |
| | Inflammation ↘ | Adult rat brain | [46] |
| | iNOS (mRNA/protein) ↘ | Adult rat brain, astrocytes, microglia | [45,46] |
| | M-CSF (mRNA) ↘ | Astrocytes | [49] |
| | TNF-α (mRNA) ↘ | Astrocytes | [49] |
| | TGF-β (mRNA/protein) ↗ | Adult mice brain | [70] |
| | IL-4 (mRNA/protein) ↗ | Adult mice brain | [70] |
| Neuro-oncology | Cell death/differentiation pathway ↗ | Rat glioma cells, human glioblastomas and anaplasic astrocytomas | [58–61,66] |
| | p75 (mRNA/protein) ↗ | Rat glioma cells | [62] |
| | Tenascin-C (mRNA/protein) ↘ | Rat glioma cells | [64] |
| | M-CSF (mRNA) ↗ | Rat glioma cells | [49] |
| | LIF (mRNA) ↗ | Rat glioma cells | [49] |

[a]Abbreviations: 1,25-$(OH)_2D_3$, 1α,25-dihydroxyvitamin $D_3$; ChAT, choline acetyl transferase; EAE, experimental autoimmune encephalomyelitis, γ-GT, γ-glutamyl transpeptidase; GDNF, glial cell line-derived neurotrophic factor; GSH, glutathione; iNOS, inducible nitric oxide synthase; IL-4, interleukin 4; LIF, leukemia inhibitory factor; L-VSCC, L-type voltage sensitive $Ca^{2+}$ channel; M-CSF, macrophage colony-stimulating factor; NGF, nerve growth factor; NT-3, neurotrophin 3; NT-4, neurotrophin 4; 6-OHDA, 6-hydroxydopamine; 24-OHase, vitamin $D_3$ 24-hydroxylase; p75, low-affinity NGF receptor; TGF-β, transforming growth factor β; TH, tyrosine hydroxylase; TNF-α, tumor necrosis factor α; VDR, vitamin D receptor.

[b]Summary of the wide range of action of 1,25-$(OH)_2D_3$ on various central or peripheral nervous system targets under normal or pathological conditions. The up arrows indicate positive regulator effects and the down arrows negative regulator effects of the hormone.

### *Multiple sclerosis, autoimmune and inflammatory disease of the CNS*

1,25-$(OH)_2D_3$ has long been known as a preventive factor for EAE [67], an animal model of the human autoimmune demyelinating CNS disease multiple sclerosis. It has recently been reported that it can reversibly block the progression of EAE when administered after the onset of clinical signs in both rats [68] and mice [69]. Thus, vitamin D deprivation aggravates the clinical symptoms of EAE in mice [69]. In these models, the beneficial effect of hormone treatment is accompanied by an inhibition of CD4 antigen expression [68], of interleukin 12 (IL-12)-dependent T helper type 1 cell development [71] and of iNOS synthesis [45] within the CNS. Similarly, a downregulation of mRNA encoding iNOS and the protein itself by 1,25-$(OH)_2D_3$ was demonstrated in a rat model of hippocampal inflammation [46]. By contrast, after 1,25-$(OH)_2D_3$ curative treatment of EAE, levels of the two anti-encephalitogenic cytokines TGF-β and IL-4 were increased in the mouse model [70]. Cells of the immune system patrolling the CNS might represent potential targets for 1,25-$(OH)_2D_3$ in immune or inflammatory diseases of the brain, but constituent cells of the CNS (such as microglial cells and astrocytes) also respond to the hormone during EAE or brain inflammation [45,46,68]. These neuro-immunomodulatory effects of 1,25-$(OH)_2D_3$ within the CNS suggest a possible beneficial effect of this hormone and of its analogs in resolving multiple sclerosis [72,73].

### *Cerebral ischemia*

Pretreatment for eight days with 1,25-$(OH)_2D_3$ reduced the amount of infarction induced by middle cerebral arterial ligation in adult rats. This effect correlated with significantly increased GDNF levels and was therefore assumed to be mediated via the upregulation of this neurotrophin [40].

### *Neurodegenerative diseases*

A reduction in *VDR* mRNA levels was detected in Alzheimer hippocampal CA1 and CA2 pyramidal

cells [16]. This observation raises the possibility that vitamin D-dependent neurotrophin synthesis or detoxification pathways could be locally altered in a minor population of neuronal cells.

### *Diabetic peripheral neuropathy*

In the experimental model of streptozotocin-diabetic rats, in which deficiencies in NGF synthesis have been reported, treatment of the animals with a 1,25-$(OH)_2D_3$ analog increases NGF production and prevents neurotrophic deficits [41].

## Concluding remarks

Currently available data strongly suggest a role for 1,25-$(OH)_2D_3$ in the nervous system. However, patients suffering from rickets show no obvious nervous dysfunction. In the same way, no gross abnormalities were identified in the brain of VDR- or 25-hydroxyvitamin D 1α-hydroxylase-deficient mice [74–76]. Even though the nervous systems of these knockout mice require more detailed study, this suggests that 1,25-$(OH)_2D_3$ functions might overlap with other signaling pathways. For instance, it is well known that NGF synthesis can be regulated independently of the presence of 1,25-$(OH)_2D_3$ [77]. Moreover, the role of 1,25-$(OH)_2D_3$ might not necessarily be apparent in these well-protected knockout mice in the absence of the above-mentioned pathogenic processes. Hence, much effort is still required to obtain a comprehensive view of the action of 1,25-$(OH)_2D_3$ in the nervous system. Systematic analysis of genes regulated by 1,25-$(OH)_2D_3$ in the nervous system; investigations of possible crosstalk among 1,25-$(OH)_2D_3$, retinoic acid, thyroid hormone and neurosteroids during neurogenesis; the elucidation of the mechanisms involved in the neuroprotective effects of this hormone; and the evaluation of the clinical relevance of 1,25-$(OH)_2D_3$ analogs to diseases, such as multiple sclerosis or glioma, are some of the areas of future research that might yield exciting results concerning the role of vitamin D in the brain. In this regard, it is noteworthy that progress in our knowledge of vitamin D biology has been punctuated with successive reappraisals of initial concepts. Thus, since the discovery of the effects of 1,25-$(OH)_2D_3$ on its classic targets tissues (intestine, bone and kidney) to control $Ca^{2+}$ homeostasis, non-classic targets (such as the brain) and non-conventional modes of action (non-genomic, ligand-independent activation) [78] have also been discovered. One of the most firmly established concepts is that of the skin as the only source of vitamin D synthesis following exposure to sunlight. However, it would be interesting to question the assumption that vitamin D synthesis occurs exclusively in the skin in view of the fact that eyes are also exposed to sunlight. This could lead to a season-dependent synthesis of vitamin $D_3$ in the eyes, followed by the axonal transport of the prehormone to specific brain areas and by its neuronal metabolism, which, if it was demonstrated, could have behavioral and psychological consequences [79–81].

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