Vitamin D, a neuro-immunomodulator: Implications for neurodegenerative and autoimmune diseases

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Received 27 March 2009; received in revised form 20 May 2009; accepted 20 May 2009

Summary It has been known for more than 20 years that vitamin D exerts marked effects on immune and neural cells. These non-classical actions of vitamin D have recently gained a renewed attention since it has been shown that diminished levels of vitamin D induce immune-mediated symptoms in animal models of autoimmune diseases and is a risk factor for various brain diseases. For example, it has been demonstrated that vitamin D (i) modulates the production of several neurotrophins, (ii) up-regulates Interleukin-4 and (iii) inhibits the differentiation and survival of dendritic cells, resulting in impaired allo-reactive T cell activation. Not surprisingly, vitamin D has been found to be a strong candidate risk-modifying factor for Multiple Sclerosis (MS), the most prevalent neurological and inflammatory disease in the young adult population.

Vitamin D is a seco-steroid hormone, produced photochemically in the animal epidermis. The action of ultraviolet light (UVB) on 7-dehydrocholesterol results in the production of pre-vitamin D which, after thermo-conversion and two separate hydroxylations, gives rise to the active 1,25-dihydroxyvitamin D. Vitamin D acts through two types of receptors: (i) the vitamin D receptor (VDR), a member of the steroid/thyroid hormone superfamily of transcription factors, and (ii) the MARRS (membrane associated, rapid response steroid binding) receptor, also known as Erp57/Grp58.

In this article, we review some of the mechanisms that may underlie the role of vitamin D in various brain diseases. We then assess how vitamin D imbalance may lay the foundation for a range of adult disorders, including brain pathologies (Parkinson’s disease, epilepsy, depression) and immune-mediated disorders (rheumatoid arthritis, type I diabetes mellitus, systemic lupus erythematosus or inflammatory bowel diseases). Multidisciplinary scientific collaborations are now required to fully appreciate the complex role of vitamin D in mammal metabolism.

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1. Introduction

Vitamin D was first discovered during the industrial revolution, when England was struck by an unprecedented epidemic of rickets. In 1918, Sir Edward Mellanby demonstrated...
that the disease was caused by a nutritional deficiency and, soon after, rachitic infants were cured with cod liver oil. 1,25-(OH)$_2$D (1,25-dihydroxyvitamin D), the active compound, was isolated for the first time, in 1922, by McCollum and was named vitamin D. Two years later, researchers from three universities discovered simultaneously that sunlight was a source of vitamin D (Hess, 1924; Hume and Smith, 1924; Steenbock, 1924). In 1965, R.B. Woodward was awarded a Nobel Price for having synthesised vitamin D and vitamin B12.

For historical and epidemiological reasons, vitamin D has been classified as a vitamin. However its synthesis from precursor molecules actually begins in skin cells. Vitamin D is now being reconsidered as a genuine steroid hormone with a multifaceted function.

2. The steroid hormone of sunlight

Vitamin D is a steroid with a broken ring and, as such, is named a seco-steroid. Vitamin D$_2$ (ergocalciferol) and vitamin D$_3$ (cholecalciferol) are the two major forms of vitamin D. Vitamin D$_2$ is derived from plants while vitamin D$_3$ is produced photochemically in the animal epidermis. The action of UVB radiation (295—310 nm) on 7-dehydrocholesterol results in the production of pre-vitamin D which, after thermo-conversion and two separate hydroxylations (performed by the P450 enzymes 25-hydroxylase and 1α-hydroxylase, respectively), gives rise to the active 1,25-(OH)$_2$D. Vitamin D synthesis peaks at wavelengths between 295 and 297 nm (UV index greater than 3) and satisfactory amounts of vitamin D are produced after 15 min of sun exposure, at least twice a week. When exposed to UVB rays during a longer period, the body degrades pre-vitamin D as fast as it generates it and equilibrium is achieved.

1,25-(OH)$_2$D can be considered as an hormone which is released into the circulation and, with the assistance of vitamin D binding protein (VDBP), is transported to various target organs. It is generally appropriate to consider this molecule as a vitamin since, in temperate regions, UVB radiation fail to provide adequate amounts of vitamin D synthesis from mid-Autumn to mid-Spring. Worldwide, a high prevalence of hypovitaminosis D among apparently healthy children, adolescents and adults has been observed (Gordon et al., 2004; Holick, 2006; Huh and Gordon, 2008; Looker et al., 2002; Rajakumar et al., 2007) and it is suggested that up to 1 billion people may have vitamin D deficiency or insufficiency (Holick, 2007). Accordingly, hypovitaminosis D is relatively common in developed countries, such as the US and the UK (Compston and Coles, 2008; Lawson et al., 1999; Thomas et al., 1998; Utiger, 1998). A large epidemiologically based US study reported that, of the women aged 20—39 (peak ages for child-bearing), 12% had low serum 25-hydroxyvitamin D levels (Looker and Gunter, 1998). In France, it has been observed that, between November and April, diet failed to provide an adequate amount of vitamin D to normal adults living in an urban environment with a lack of direct exposure to sunshine (Chapuy et al., 1997). Pregnant women are at risk of hypovitaminosis D, especially those wearing concealing clothes (Belaid et al., 2008), because of the increased needs of the foetus and the potential for these women to reduce their outdoor activity, leading to a diminished supply of vitamin D (Hillman and Haddad, 1976; Markestad et al., 1983). Of great concern is the observation that at the end of spring, one baby out of four shows signs of hypovitaminosis D (Zeghoud et al., 1997).

Initially, it was thought that liver and kidneys were the only organs responsible for the production of 1,25-(OH)$_2$D. However, it is now clearly established that many tissues, including the brain (Eyles et al., 2005), express vitamin D 1α-hydroxylase, the limiting enzyme responsible for the formation of 1,25-(OH)$_2$D. 1,25-(OH)$_2$D receptors are also found throughout the whole body including the CNS.

Like other neurosteroids (i.e. oestrogen) 1,25-(OH)$_2$D is believed to act via two types of receptors: (i) the nuclear vitamin D receptor (VDR), a member of the steroid/thyroid hormone super-family of transcription regulation factors, and (ii) the putative membrane receptor—MARRS (membrane associated, rapid response steroid binding), also known as Erp57/Grp58. The classical actions of vitamin D start with binding to the VDR which, in turn, hetero-dimerises with nuclear receptors of the retinoic X receptor (RXR) family binding to vitamin D responsive elements (VDRE), located in the promoter regions of hundreds of target genes (Wang et al., 2005). The more rapid non-classical actions of 1,25-(OH)$_2$D are believed to involve binding to the MARRS receptor, located at the cell surface, initiating non-genomic effects such as the rapid stimulation of calcium and phosphorus uptake (Khanal and Nemere, 2007).

2.1. Genomic effects: the vitamin D receptor, a transcription factor

Upon 1,25-(OH)$_2$D binding, the VDR is phosphorylated and recruits one of the three 9-cis retinoid X receptors (RXR). Modulation of gene expression is then dependent of the ability of these hetero-dimers to recruit co-regulatory proteins complexes including the steroid receptor co-activators (SRCs) and the vitamin D receptor interacting protein (DRIP). These complexes bind to specific genomic sequences named vitamin D responsive elements (VDRE). The VDR not only directly activates gene transcription but also directly down-regulates the transcription of several genes such as those encoding PTH or CYP27B1 (for reviews, see Bouillon et al., 2008; Kato et al., 2007; McDonald, 1984) (Fig. 1).

2.2. Rapid non-genomic effects

In addition to genomic effects, 1,25-(OH)$_2$D like other neurosteroids mediates these effects through rapid non-genomic actions. Thus, 1,25-(OH)$_2$D activates a variety of signal transduction systems including Ca$^{2+}$ influx, release of Ca$^{2+}$ from intracellular stores, modulation of adenylate cyclase, PLC and protein kinases C and D as well as MAP and Raf kinase pathways. These activities have been observed in many cells including enterocytes, keratinocytes, muscle cells, osteoblasts, chondrocytes and are cell type dependent (for reviews see Falkenstein et al., 2000). VDR is necessary for some of these non-genomic pathways, however another protein named 1,25-(OH)$_2$D-MARRS is also involved in these rapid non-genomic actions (Khanal et al., 2008; Nemere et al., 2004; Rohe et al., 2005; Teillaud et al., 2005) (Fig. 1).
3. A neuro-immuno-modulator

Over the past 15 years accumulating data have provided evidence that targets of 1,25-(OH)₂D are multiple (Holick, 2006; Szodoray et al., 2008) and include nervous system tissues (Buell and Dawson-Hughes, 2008; Cherniack et al., 2009; Kiraly et al., 2006; McCann and Ames, 2008).

3.1. Vitamin D and the nervous system

Vitamin D receptors (VDR) are widely distributed throughout the embryonic brain prominently in the neuro-epithelium and proliferating zones (Stumpf et al., 1982). Expression is not confined to these regions; VDR is expressed widely in the adult brain in temporal, orbital and cingulate cortex, in the thalamus, in the accumbens nuclei, parts of the stria terminalis and amygdala and widely throughout the olfactory system. It is also expressed in pyramidal neurons of the hippocampal regions CA1, CA2, CA3, CA4, in rats (Stumpf et al., 1982) as well as in humans (Eyles et al., 2005).

In parallel, it has been shown that (i) 1,25-(OH)₂D is present in the cerebrospinal fluid (Balabanova et al., 1984) and (ii) genes coding for the enzymes involved in the biosynthesis/catabolism of this hormone are expressed in the brain (Eyles et al., 2005; Naveilhan et al., 1993; Neveu et al., 1994b).

Local synthesis of 1,25-(OH)₂D is performed by 1α-hydroxylase-expressing neurons and microglia. Altogether, these findings suggest that 1,25-(OH)₂D could act in an autocrine/paracrine fashion in nervous system (for review see Garcia et al., 2002; Kalueff and Tuohimaa, 2007).

Fig. 1 Vitamin D genomic and non-genomic signaling pathways. 1,25-(OH)₂D binds to its nuclear receptor (VDR) which, after heterodimerisation with RXR, induces genomic responses. The 1,25D/VDR/RXR complex recognises vitamin D responsive elements (VDRE) within the promoter region of hundreds of genes. Upon binding, co-repressor factors (CoRe) or co-activator factors (CoAc) are recruited. Genes whose expression is repressed by 1,25-(OH)₂D comprise parathyroid hormone (PTH) and 1-α-hydroxylase. Within the immune and the nervous systems, genes with an enhanced expression include (i) osteopontin, cathelicidin, TNF alpha, HLA DRB15* and (ii) NGF, p75NTR, TGF beta, calbindin, respectively. 1,25-(OH)₂D can also bind to its membrane receptor (MARRS) and induce rapid non-genomic responses. 1,25-(OH)₂D regulates the activity of adenylate cyclase, phospholipase C (PLC), protein kinase C (PKC), Src proteins. 1,25-(OH)₂D induces the release of Ca²⁺ from intracellular stores as well as the recruitment of extracellular Ca²⁺ through store operated channels (SOC). 1,25-(OH)₂D also modulates the cell cycle via TGF and EGF receptors. When located in the endoplasmic reticulum, MARRS is involved in MHC1 assembly. VDR: Vitamin D Receptor; RXR: Retinoic acid X Receptor; MARRS: membrane associated, rapid response steroid binding; TNF: Tumour Necrosis Factor; NGF: Nerve Growth Factor; p75NTR: low affinity neurotrophin receptor; TGF: Transforming Growth Factor; EGF: Epidermal Growth Factor; MHC I: major histocompatibility complex class I.
cell line-derived neurotrophic factor (GDNF) (Naveilhan et al., 1993) whereas neurotrophin 4 (NT4) was down-regulated (Neveu et al., 1994b) in glioma cell lines. In addition, vitamin D increases neurite outgrowth, when added to cultured hippocampal cells (Brown et al., 2003). Conversely, when vitamin D is removed from the diet of pregnant rat females, decreased expression of NGF is observed in the brains of both, neonates (Eyles et al., 2003) and adult offspring (Feron et al., 2005).

Vitamin D may also affect neuronal plasticity processes such as axogenesis. It has been shown that vitamin D up-regulates the expression of microtubule-associated protein-2 (MAP2), growth-associated protein-43 (GAP43) and synapsin-1 in cultured rat cortical neurons (Taniura et al., 2006). In parallel, using a rat model of maternal hypovitaminosis D, we demonstrated a robust and consistent down-regulation of transcripts and proteins involved in cytoskeleton maintenance (neurofilament, tubulin, actin, MAP2, glial fibrillary acidic protein ...), molecular transport of organelles (creatine kinase b, kinesin, RhoA, dynactin) and synaptic plasticity (drebrin, GAP43, connexin 43) (Almeras et al., 2007; Eyles et al., 2007).

Finally, vitamin D has been shown to be neuro-protective, notably by inducing the synthesis of Ca<sup>2+</sup>-binding proteins, such as parvalbumin (de Viragh et al., 1989). Vitamin D has also been reported to inhibit the synthesis of inducible nitric oxide synthase (Garcion et al., 1997, 1998), an enzyme induced in neurons and non-neuronal cells during ischemia or in the neurodegenerative conditions Alzheimer’s disease, Parkinson’s disease, AIDS, infections, multiple sclerosis). These actions are summarised in Fig. 2.

3.2. Vitamin D and the immune system

It has been known for more than 20 years that vitamin D exerts marked effects on immune cells (Lemire et al., 1984; Rigby et al., 1984). However, this non-classical action of vitamin D has recently gained a renewed attention since it has been shown that diminished vitamin D (i) induces immune-mediated symptoms in animal models of autoimmune diseases such as rheumatoid arthritis, type I diabetes mellitus, systemic lupus erythematosus or inflammatory bowel diseases (for a review Szodoray et al., 2008) and (ii) is a risk factor for viral infections, including tuberculosis (Liu et al., 2006) and possibly influenza (Cannell et al., 2006). It has also been demonstrated that vitamin D reverses age-related inflammatory changes in the rat hippocampus (Moore et al., 2005).

Macrophages and some dendritic cells express the VDR as well as the two cytochrome P450 enzymes required to produce vitamin D. Activated T cells and, possibly, B cells express 1α-hydroxylase and VDR only after activation (Moro et al., 2008). CD4-positive T lymphocytes (Th1, Th2, Th17) have been shown to be the preferential target of vitamin D. 1,25-(OH)₂D inhibits
Th1 cells and the production of Th1 cytokines like IL-2, IFN-γ, and TNF-α (Lemire and Archer, 1991).

Vitamin D affects the differentiation and function of cells in the immune system. CD4-positive T lymphocytes have been shown to be the preferential target of vitamin D. The three distinct functional CD4 cell types are Th1, Th2, and Th17 cells. Th1 cells preferentially produce IL-2, IFN-γ, and TNF-α and stimulate the cellular immune system. Th2 cells principally secrete IL-4 and IL-10 and inhibit Th1 function. Th17 cells predominantly express IL-17. Th1 cells are the main effector cells of a number of autoimmune diseases and of organ rejection (Liblau et al., 1995). It has been demonstrated that vitamin D inhibits Th1 cells and the production of Th1 cytokines like IL-2, IFN-γ, and TNF-α (Lemire and Archer, 1991). It has been previously observed that the mechanism involves a VDR mediated inhibition of gene transcription (Alroy et al., 1995; Cippitelli and Santoni, 1998). Vitamin D has also been reported to (i) up-regulate IL-4 (Cantorna et al., 1998) and IL-10 (Correale et al., 2009) and (ii) inhibit the production of IL-6 and IL-17 (Correale et al., 2009) as well as the differentiation and survival of dendritic cells, resulting in impaired allo-reactive T cell activation (Cantorna, 2006; Griffin et al., 2001; Mathieu and Asorini, 2002).

Vitamin D has long been known as a preventive factor for experimental autoimmune encephalomyelitis (EAE) (Lemire and Archer, 1991), an animal model of multiple sclerosis. Vitamin D reversibly blocks the progression of EAE when administered after the onset of clinical signs in both rats (Nataf et al., 1996) and mice (Cantorna et al., 1996). In these models, the beneficial effect of vitamin D treatment is accompanied by an inhibition of (i) CD4 antigen expression (Nataf et al., 1996), (ii) interleukin 12 (IL-12)-dependent T helper type 1 cell development (Muthian et al., 2006), (iii) iNOS synthesis within the CNS (Garcion et al., 1997) and by enhancing (i) an IL-10-dependent anti-inflammatory loop (Spach et al., 2006) and (ii) apoptotic death of inflammatory CD4+ T cells (Pedersen et al., 2007). Similarly, a down-regulation of mRNA encoding iNOS and the protein itself by vitamin D was demonstrated in a rat model of hippocampal inflammation (Garcion et al., 1998). Furthermore, after vitamin D curative treatment of EAE, levels of the anti-inflammatory cytokines TGF-β and IL-4 were increased in the mouse model (Cantorna et al., 1998). Cells of the immune system patrolling the CNS might represent potential targets for vitamin D in immune or inflammatory diseases of the brain, but microglial cells and astrocytes also respond to the hormone during EAE or brain inflammation (Garcion et al., 1997, 1998; Nataf et al., 1996). The immuno-modulatory properties of vitamin D are summarised in Fig. 3.

### 4. Vitamin D and multiple sclerosis

Multiple Sclerosis (MS), the most prevalent neurological disorder in the young adult population, is an inflammatory disease in which the immune system attacks the central nervous system (CNS). Vitamin D has been shown to play a crucial role in modulating the immune response against the CNS, thereby potentially offering therapeutic benefits in MS. The mechanisms by which vitamin D impacts MS are multifaceted and involve a variety of immune cells and cytokines.

**Vitamin D as a Neuro-Immunomodulator:**

Vitamin D modulates the immune response by influencing the development and function of T cells and dendritic cells. In the context of MS, vitamin D has been observed to down-regulate the production of pro-inflammatory cytokines such as IL-2, IFN-γ, IL-16, IL-23, and TNF-α, and up-regulate anti-inflammatory cytokines such as IL-4, IL-5, IL-10, and IL-16. This balance shift is thought to be beneficial in reducing the inflammatory burden associated with MS.

**Tissue-Specific Effects:**

Vitamin D has been shown to influence multiple sclerosis through its effects on various immune cells. For instance, it can inhibit the recruitment of CD4+ lymphocytes by inhibiting the production of IL-12 by macrophages and DCs, thereby reducing the activation of Th1 cells. Additionally, it enhances the differentiation of Th2 cells, which are associated with an anti-inflammatory response.

**Regulation of Inflammatory Cytokines:**

Vitamin D can regulate the expression of inflammatory cytokines. It has been observed that vitamin D increases the expression of IL-4, IL-5, and IL-10, which are associated with a Th2 phenotype, and decreases the expression of pro-inflammatory cytokines such as IL-2, IL-16, and IL-23.

**Impacts on Microglia and Astrocytes:**

Microglia and astrocytes, which are resident cells of the CNS, are also responsive to vitamin D. Vitamin D has been shown to down-regulate the expression of iNOS (inducible nitric oxide synthase) in these cells, which is a key mediator of inflammation.

**Fig. 3: Vitamin D and the immune system.**

Vitamin D and its metabolic products, such as 1,25-dihydroxyvitamin D3 (1,25-(OH)2D), play a crucial role in the modulation of the immune response. They can act directly on immune cells or indirectly through the activation of transcription factors like T-bet, which regulates the expression of Th1-associated genes.

**Vitamin D and the Immune Response:**

- **1-OHase:** 1-OHase is involved in the metabolism of vitamin D, converting it to its active form, 1,25-(OH)2D.
- **VDR:** Vitamin D receptor (VDR) is expressed on various immune cells, including macrophages, dendritic cells, and CD4+ cells.
- **MARRS:** MARRS is ubiquitously produced and plays a role in the regulation of immune cell function.
- **TLR1/2:** Toll-like receptors (TLR1/2) are activated by vitamin D through the production of the antibiotic cathelicidin.
- **CD4, CD80, CD86:** These are co-stimulatory molecules expressed on dendritic cells.
- **CD4+**: CD4+ cells are a crucial component of the immune response and are targeted by vitamin D.
- **Act B:** Activated B cells (Act B) can be regulated by vitamin D.
- **CD80, CD86:** These costimulatory molecules are associated with T cell activation.
- **NK:** Natural killer (NK) cells are also responsive to vitamin D, with increased expression of NK1.1, a marker for NK cell maturation.
- **T-bet factor:** T-bet, a transcription factor, is upregulated by vitamin D in NK cells.
- **1,25-(OH)2D:** 1,25-dihydroxyvitamin D3 is the active form of vitamin D, playing a key role in immune modulation.
- **TGF-β:** Transforming growth factor-β, an anti-inflammatory cytokine, is increased in response to vitamin D treatment.
- **IL-4, IL-5, IL-10, FoxP3:** Antigen-specific Th2 cells are associated with the production of these cytokines.
- **Th1, Th2:** Th1 and Th2 cells represent the two major T helper subsets, with Th1 cells being associated with disease and Th2 cells with resolution.
- **CD4+ FoxP3+ Treg cells:** Regulatory T cells (Treg) are critical for maintaining immune tolerance.
- **NF-kB:** Nuclear factor-kappa B, a transcription factor, is involved in immune cell activation and is regulated by vitamin D.
nervous system, provoking demyelination and axon degeneration (Compston and Coles, 2008). Approximately 15–20% of MS patients have a family history of MS and studies in twins (Ebers et al., 1986; Heltberg et al., 1985; Kinnunen et al., 1987; Mackay and Myrianthopoulos, 1966; Mumford et al., 1994; Williams et al., 1980) and conjugal pairs (Robertson et al., 1997) indicate that much of this familial clustering is the result of shared genetic risk factors. To date, the Major Histocompatibility Complex (MHC) gene region is the preponderant area of the human genome associated with the disease. MS is also prompted by environmental exposure.

Goldberg was the first to link sunlight, dietary factors and epidemiology of MS (Goldberg et al., 1986). He surmised that MS could result from an inadequate supply of vitamin D and calcium at times of rapid myelination, mainly in adolescence. Thirty-five years later, epidemiological, animal and in vitro data are compelling and indicate that low vitamin D is a candidate risk-modifying factor for MS.

1. Latitude variations. The disease is virtually unknown in equatorial regions and there is an inverse correlation between latitude (a proxy marker for vitamin D levels) and disease prevalence (Esparza et al., 1995; Giovannoni and Ebers, 2007; Hammond et al., 2000; Hernan et al., 1999; Kurtzke et al., 1997; McGuigan et al., 2004; Norman et al., 1983; Weinschenker, 1996). This inverse correlation with latitude is also present within countries being observed in Australia (Hammond et al., 2000; McLeod et al., 1994), Canada (Willer et al., 2005), France (Vukusic et al., 2007), New Zealand (Fawcett and Skegg, 1988) and the USA (Templer et al., 1992).

2. Ultraviolet B radiation (UVB). MS prevalence increases with decreasing solar radiation, suggesting a protective effect of sunlight. A reduced risk of MS was associated with (i) higher sun exposure when aged 6–15 years in Tasmania (van der Mei et al., 2003), (ii) summer outdoor activities in childhood and adolescence in Norway (Kampman et al., 2007) and (iii) sun sensitive skin types 1 and 2 in the UK (Woolmore et al., 2007). In the USA, a prospective study among more than 7 million military personnel suggest that high circulating levels of vitamin D is correlated to a lower risk of MS (Munger et al., 2006). Furthermore, incidence of demyelination in MS parallels seasonal fluctuations in vitamin D levels (Auer et al., 2000).

3. Season of birth. There is evidence in many areas that MS has seasonality of birth (Acheson et al., 1960; Fernandes de Abreu et al., in press; Leibowitz et al., 1968; Sutherland et al., 1962; Templer et al., 1992; Torrey et al., 2000).

4. Oral vitamin D intake. Areas with diets rich in fish oil (a major source of vitamin D) have lower incidence of MS (Agranoff and Goldberg, 1974; Hayes et al., 1997). A protective effect of vitamin D intake on risk of developing MS has been reported in a cohort of nearly 200,000 women (Munger et al., 2006).

5. In vitro and animal experiments. As previously stated, vitamin D and analogues ameliorate the clinical outcome in EAE animal model of MS. When administered during the immunisation phase, vitamin D prevents clinical signs of EAE. When vitamin D is given after the beginning of clinical signs, a significant improvement is observed (Cantorna et al., 1996; Garcia et al., 2003; Meehan and DeLuca, 2002; Nashold et al., 2001, 2000; Nataf et al., 1996; Spach and Hayes, 2005; Spach et al., 2006, 2004). It has been observed that vitamin D reverses EAE by inhibiting chemokine synthesis and monocyte trafficking (Pedersen et al., 2007). Conversely, a post-natal vitamin D deficiency induces an earlier onset of the EAE symptoms (Cantorna et al., 1996; Garcia et al., 2003) and an amplified severity of the symptoms, including a second paralytic attack with a noticeable ataxia (Garcia et al., 2003).

So far, very few clinical trials in MS patients have been conducted. Dietary supplementation with vitamin D and other nutrients was found to decrease relapse rate in two small groups of young MS patients but methodological biases cast a doubt on the conclusions (Goldberg et al., 1986; Nordvik et al., 2000). More recently, a small safety study in 12 patients, supplemented with vitamin D, reported a decline in the number of demyelinating plaques but without any observed symptom improvement (Kimball et al., 2007). Larger well-controlled trials are needed.

5. Mechanisms of action for vitamin D and multiple sclerosis

No undisputed molecular mechanism underlying the role of vitamin D in MS has been unveiled so far. However, several metabolic pathways, possibly complementary, can be proposed.

1. Vitamin D induces naïve CD4+ T to differentiate into regulatory T cells producing IL-10. These cells are able to prevent CNS inflammation when they are targeted to the site of inflammation (O’Garra and Barret, 2003). Accordingly, it has been found that IL-10 is essential for vitamin D-mediated inhibition of EAE (Spach et al., 2006). In addition, vitamin D stimulates NK cells that are known to play an immuno-regulatory role in the prevention of autoimmune diseases (Baxter and Smyth, 2002). Deficiencies in NK cells are described in patients with MS (Takahashi et al., 2004, 2001), or other autoimmune diseases such as systemic lupus erythematosus and type 1 diabetes mellitus.

2. Vitamin D is a tissue-specific stimulator or inhibitor of osteopontin, a molecule named due to its role in ossification (Broess et al., 1995; Reinhold et al., 1990; Yoon et al., 1987). However, osteopontin is also a pro-inflammatory cytokine, with a pleiotropic action on the immune system. Osteopontin (i) increases IL-12 production by macrophages; (ii) enhances interferon gamma and TNF expression by T cells; (iii) blocks IL-10 production by B cells and Treg cells and (iv) augments survival of activated T cells (Stromnes and Goverman, 2007). Interestingly, osteopontin-deficient mice are resistant to EAE and have frequent remissions (Chabas et al., 2001). Moreover, an increased number of osteopontin transcripts are also found in the brain of MS patients (Chabas et al., 2001).

3. In one of our previous studies, we demonstrated that a prenatal vitamin D deficiency led to a permanent dysre-
gulation of many transcripts, including calcineurin and one of the FK506 binding proteins (FKBP) (Eyles et al., 2007) in brain tissue of offspring. These two molecules play a pivotal role in immuno-suppression by limiting the production of IL-2, which is necessary for full T-cell activation. When bound to its ligand, FKBP blocks the function of the enzyme calcineurin and, as a consequence, inhibits the activation of the cytoplasmic component of the nuclear factor of activated T cells (NF-ATc), preventing its entrance into the nucleus (Stepkowska, 2000).

4. We also observed that a prenatal vitamin D deficiency induced an altered expression of transcripts and proteins such as catalase, calxin, various Heat Shock Proteins (HSP) and GRP58/ERp57, which is also known as MARSS, the putative membrane vitamin D receptor (Almeras et al., 2007; Eyles et al., 2007). Interestingly, these molecules are involved in antigen presentation and the transport of steroid receptor from the cytoplasm to the nucleus (Desjardins, 2003). In addition, GRP58/ERp57/MARRS interacts with MHC I molecules and therefore may play an unrecognized role in adaptive immunity.

5. The Major Histocompatibility Complex (MHC) gene region is the preponderant area of the human genome associated with multiple sclerosis. Within this region, HLA-DRB1*1501 is the major locus determining genetic susceptibility for MS (ref). Attractively, it has been shown very recently that vitamin D directly regulates the expression of this gene (Ramagopalan et al., 2009).

6. It has been proposed that inadequate supply of vitamin D during developmental myelination, mainly in adolescence, is a risk factor for MS (Goldberg, 1974). This theory is partly substantiated by a study showing that oligodendrocytes express VDR and respond to 1,25-(OH)2D (Baas et al., 2000). In addition, we have shown that a prenatal vitamin D deficiency induces a permanent down-regulation of Myelin-associated oligodendrocytic basic protein (MOBP) mRNA within the cerebrum of the adult offspring (Eyles et al., 2007). However, it remains to be demonstrated that vitamin D regulates the expression of myelin proteins.

6. Implications for other brain diseases

The widespread expression of the receptor for vitamin D and enzymes responsible for its synthesis in the CNS suggest that reductions in this hormone production may be relevant for a number of neurodegenerative or psychiatric pathologies.

6.1. Parkinson’s disease

The substantia nigra (the portion of the brain that degenerates in Parkinson’s disease) represents the area of the human brain where the VDR is most highly expressed (Eyles et al., 2005). Vitamin D also (i) protects in vitro mesencephalic neurons from Parkinson-like insults (Shinpo et al., 2005) and (ii) reduces the lesion induced by injection of 6-hydroxy-dopamine (Smith et al., 2006; Wang et al., 2001). Vitamin D Responsive Elements (VDRE) have been identified in silico in the promoter regions of GDNFR-α, ret, and neurturin, three genes strongly linked to Parkinson’s disease ((Wang et al., 2005 supplementary materials) and it has been demonstrated that vitamin D positively regulates the expression of GDNF (Sanchez et al., 2002) and tyrosine hydroxylase (Puchacz et al., 1996). Furthermore, a permanent down-regulation of Park 7 expression has been described in the hippocampus of adult rats born to vitamin D-deficient mothers (Eyles et al., 2007).

Confirmatively, a significant higher prevalence of hypovitaminosis D was observed in patients with Parkinson’s disease, when compared to both healthy controls and patients with Alzheimer’s disease (Evatt et al., 2008). In addition, a positive association between VDR gene polymorphism and Parkinson’s disease has been reported (Kim et al., 2005). Finally the vitamin D binding protein (VDBP) has been recently shown to be one of eight cerebrospinal fluid biomarkers in Parkinson’s disease (Zhang et al., 2008).

6.2. Epilepsy

A neuro-protective effect of vitamin D in a rodent model of epilepsy was unveiled in 1984. When vitamin D is delivered within the hippocampus, the threshold for provoked seizures is reduced (Siegel et al., 1984). More recently, Kalueff’s team has demonstrated that vitamin D plays an anti-convulsive role in an epilepsy animal model, triggered by pentylenetetrazole chloride (PTZ). Injection of vitamin D, 30—180 min before seizure induction, reduces the deleterious effect of PTZ (Kalueff et al., 2005). Similarly, administration of PTZ to VDR KO mice induces shorter latencies to the onset and increased mortality rates (Kalueff et al., 2006).

An increased expression of VDR within the hippocampus has been observed in rats after pilocarpine-induced seizures (Janjoppi et al., 2008). In parallel, proteomic analysis of cerebrospinal fluid (CSF) from patients with temporal lobe epilepsy (TLE) and controls indicated an elevated expression of vitamin D-binding protein (DBP) (Xiao et al., 2009).

6.3. Depression

Vitamin D Responsive Elements have been identified in silico in the promoter regions of serotonin receptors and tryptophan hydroxylase, two genes associated with depression ((Wang et al., 2005) supplementary materials). It is also known that vitamin D protects against serotonin-depleting effects of neurotoxic doses of methamphetamine (Cass et al., 2006). Finally, in a very large study involving more than 1000 older adults, mean levels of 25-hydroxyvitamin D were found significantly lower in those with minor depression and major depression when compared to controls (Hoogendijk et al., 2008).

6.4. Schizophrenia

The idea that low prenatal vitamin D could be a risk factor for the adult onset of schizophrenia was first proposed in 1999 (McGrath, 1999). Many studies have shown that those born in winter and spring have a significantly increased risk of developing schizophrenia (Torrey and Miller, 1997). This risk is magnified with increasing latitude (Davies et al., 2003). The incidence and prevalence of schizophrenia is also greater from sites at higher latitudes (Saha et al., 2006).
The incidence of schizophrenia is also significantly higher in dark-skinned migrants to cold countries compared to the native born (Cantor-Graae and Selten, 2005). Given that hypovitaminosis D is more common (a) during winter and spring, (b) at high latitudes, and (c) in dark-skinned individuals (Holick, 2005), low prenatal vitamin D ‘fits’ these key environmental features. Preliminary evidence from analytical epidemiology studies also suggests that prenatal vitamin D warrants closer attention as a candidate risk factor. For example, vitamin D supplements in the first year of life significantly reduced the risk of schizophrenia in males from a large Finnish birth cohort (McGrath et al., 2004). In addition, a pilot study found that 25-hydroxyvitamin D₃ serum levels in 26 mothers whose children developed schizophrenia were lower than that of 51 control mothers whose children did not develop the disease, but this group difference was more prominent in mothers with dark skin (McGrath et al., 2003). A model of developmental vitamin D deficiency to test this hypothesis has been developed in rodents (Eyles et al., 2003; Feron et al., 2005) and is the subject of another article in this issue.

7. Implications for immune-mediated disorders

For many years, exposure to sunlight has been advocated as a mean to fight immune-mediated disorders. However, in most cases, evidence was lacking. Now, convergent studies provide a more solid background for this kind of treatment or the oral delivery of vitamin D.

7.1. Tuberculosis

In 1895, Niels Finsen was the first to expose individuals with tuberculosis — lupus vulgaris — to the rays of a mercury lamp. This simple method was efficient in curing 95% of the patients (Zasloff, 2006). “In recognition of his contribution to the treatment of diseases, especially lupus vulgaris, with concentrated light radiation”, he was awarded the Nobel Prize in Medicine and Physiology in 1903. However, this technique has not been extensively used and it is only recently that it was shown that (i) Mycobacterium tuberculosis activates the production of VDR and 1-hydroxylase (Schauber et al., 2007) and (ii) many patients with lupus vulgaris are vitamin D-deficient (Chan, 2000).

The pathogenic agent, Mycobacterium tuberculosis, binds to the Toll like receptor1/2 (TLR), expressed by monocytes and macrophages (Liu et al., 2007). When vitamin D is added to the culture medium of monocytes and macrophages infested with Mycobacterium tuberculosis, the intracellular bacterium replication is strongly reduced (Crowle et al., 1987; Rook et al., 1986). Vitamin D acts via cathelicidin, an antibiotic compound that exhibit a VDRE in its promoter region (Gombart et al., 2005). Conversely, when infested monocytes are cultivated with a VDR antagonist or in a vitamin D-free medium, cathelicidin is not expressed anymore (Liu et al., 2006).

In mice, a different response to Mycobacterium tuberculosis is at play. The activation of TLR1/2 induces an increased production of inducible nitric oxide synthase (iNOS), which also displays a VDRE in its promoter region (Liu et al., 2007). Moreover, the anti-microbial action of vitamin D on infested monocytes and macrophages entails the regulation of phosphatidylinositol-3 kinase (PI3-K) (Sly et al., 2001). The latter mechanism is mediated by a non-genomic vitamin D receptor.

7.2. Immunosuppression

Heterologous grafting of organs requires the use of immuno-suppressants. These agents work by either reducing the number of lymphocytes or blocking their metabolism. Several steroids are currently used as immuno-suppressive molecules (Hale, 2004). Among them, vitamin D stands as a strong contender. A therapeutic benefit has been observed after transplantation of (i) hearts, (ii) small intestines, (iii) livers, (iv) Langerhans islets, (v) bone marrows, (vi) skins and (vii) kidneys (Baeke et al., 2008). Vitamin D immuno-modulatory properties are mediated by T lymphocytes and dendritic cells.

7.3. Autoimmune diseases

Hypovitaminosis D is associated to a higher prevalence for a very large number of autoimmune diseases (Szodoray et al., 2008). Numerous epidemiological and animal studies indicate that, in addition to multiple sclerosis, a dysregulated vitamin D metabolism is involved in (i) diabetes mellitus type 1, (ii) inflammatory bowel diseases, (iii) rheumatoid arthritis and (iv) systemic lupus erythematosus.

7.4. Diabetes mellitus type 1

Like for multiple sclerosis, an inverse correlation between latitude and disease incidence is found for diabetes mellitus type 1 (Pozzilli et al., 2005; Svoren et al., 2009). Children with rickets have a three fold increased risk of developing diabetes mellitus type 1 (Mathieu and Adorini, 2002). Conversely, a vitamin D supplementation during childhood reduces the chance of being diabetic as an adult (The EURODIAB Substudy 2 Study Group, 1999). The animal model for this disease, named Non Obese Diabetes (NOD), mimics the main symptoms around Week 8. A peri-natal vitamin D deficiency induces an increased prevalence with an earlier onset and more severe symptoms while a postnatal vitamin D supplementation reduces Langerhans islet apoptosis and limits symptom severity (Bouillon et al., 2008).

7.5. Inflammatory bowel diseases

The main forms of inflammatory bowel diseases are Crohn’s disease and ulcerative colitis. Once again, a latitude gradient is observed for these diseases and most patients are vitamin D-deficient (Cantorna and Mahon, 2004). Interleukin-10 knockout mice exhibit most of the attached symptoms between Week 9 and Week 12 (Kuhn et al., 1993). Vitamin D-deficient IL-10 KO mice start dying at Week 6 and, at Week 9, half of them are dead (Cantorna, 2000).

7.6. Rheumatoid arthritis

A higher prevalence for this disease is associated to hypovitaminosis D (Merlino et al., 2004) and the use of alfacalcidol,
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a vitamin D analogue, improves the symptoms in most (89%) patients (Andjelkovic et al., 1999). One animal model is based on mouse immunisation with type II collagen. When administered during the immunisation phase, vitamin D prevents clinical signs of rheumatoid arthritis (Cantorna et al., 1998). When vitamin D is given after the beginning of clinical signs, the progression of the disease is blocked (Larsson et al., 1998).

7.7. Systemic lupus erythematosus

Vitamin D and some of its analogues reduce the symptoms observed in lpr/lpr knockout mice, the animal model for this disease (Abe et al., 1999). Furthermore, it has been demonstrated that vitamin D inhibits induced mitosis of B cells in asymptomatic patients but not spontaneous mitosis of B cells in symptomatic patients (Chong et al., 1989).

8. Conclusions

Vitamin D exhibits all the main characteristics of a true neuroactive steroid. We highlighted how deficiencies, prevalent all around the world, may contribute to a previously unrecognized diverse range of adverse CNS outcomes, including autoimmune and neurodegenerative diseases. It is our wish that this review will inspire clinical and basic researchers to collaborate in an effort to understand the pleiotropic roles of vitamin D in brain function.

Conflict of interest

None declared.

Acknowledgments

We gratefully acknowledge Alarime, ARSEP, Demain Debout Foundations, Fondation de l’Avenir and IRME (Institut pour la Recherche sur la Moelle épinière et l’Encéphale) and the National Health and Medical Research Council of Australia for their financial support.

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