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Vitamin D, reactive oxygen species and calcium signalling in ageing and disease

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Vitamin D is a hormone that maintains healthy cells. It functions by regulating the low resting levels of cell signalling components such as Ca^{2+} and reactive oxygen species (ROS). Its role in maintaining phenotypic stability of these signalling pathways depends on the ability of vitamin D to control the expression of those components that act to reduce the levels of both Ca^{2+} and ROS. This regulatory role of vitamin D is supported by both Klotho and Nrf2. A decline in the vitamin D/Klotho/Nrf2 regulatory network may enhance the ageing process, and this is well illustrated by the age-related decline in cognition in rats that can be reversed by administering vitamin D. A deficiency in vitamin D has also been linked to two of the major diseases in man: heart disease and Alzheimer's disease (AD). In cardiac cells, this deficiency alters the Ca^{2+} transients to activate the gene transcriptional events leading to cardiac hypertrophy and the failing heart. In the case of AD, it is argued that vitamin D deficiency results in the Ca^{2+} landscape that initiates amyloid formation, which then elevates the resting level of Ca^{2+} to drive the memory loss that progresses to neuronal cell death and dementia.

This article is part of the themed issue 'Evolution brings Ca^{2+} and ATP together to control life and death'.

1. Introduction

A large number of cellular processes are regulated by calcium (Ca^{2+}). An important component of Ca^{2+} signalling is the $InsP_3/Ca^{2+}$ signalling pathway, which has two main operational modes. It functions either as a primary signalling pathway or it can operate as a modulatory signal. Its primary role is evident mainly in non-excitable cells where it generates the Ca^{2+} signals to control processes as diverse as fertilization, proliferation, metabolism, secretion and smooth muscle contraction. In excitable cells, the primary Ca^{2+} signal depends on the entry of Ca^{2+} through voltage-operated channels and the release of Ca^{2+} by ryanodine receptors (RYRs) on the internal stores. This primary Ca^{2+} pathway regulates processes such as contraction in the heart or memory formation in neurons. The $InsP_3/Ca^{2+}$ signalling pathway provides a modulatory signal that can induce subtle changes in the generation and function of this primary Ca^{2+} signal. In this review, I will argue that subtle changes in the nature of this modulatory role of the $InsP_3/Ca^{2+}$ signalling pathway may be responsible for the onset of two major human diseases: Alzheimer's disease (AD) and cardiovascular disease.

Both cardiac disease and AD are age related and what is remarkable is their very slow progression. Most individuals who develop these diseases are completely unaware that the disease is developing, and it is this aspect that may be explained by the subtle modulatory activity of the $InsP_3/Ca^{2+}$ signalling pathway. It will be argued that one of the main causes of the alteration in this modulatory pathway is vitamin D deficiency that causes the small alterations in the Ca^{2+} signalling pathway responsible for the onset of these two diseases [1–3]. All this evidence raises a major question concerning what it is about vitamin D that makes it such an important component of a healthy life. Any hypothesis as to how vitamin D deficiency might contribute to disease has to take into account a possible relationship between ageing and vitamin D deficiency. There is increasing evidence that vitamin D acts by maintaining the

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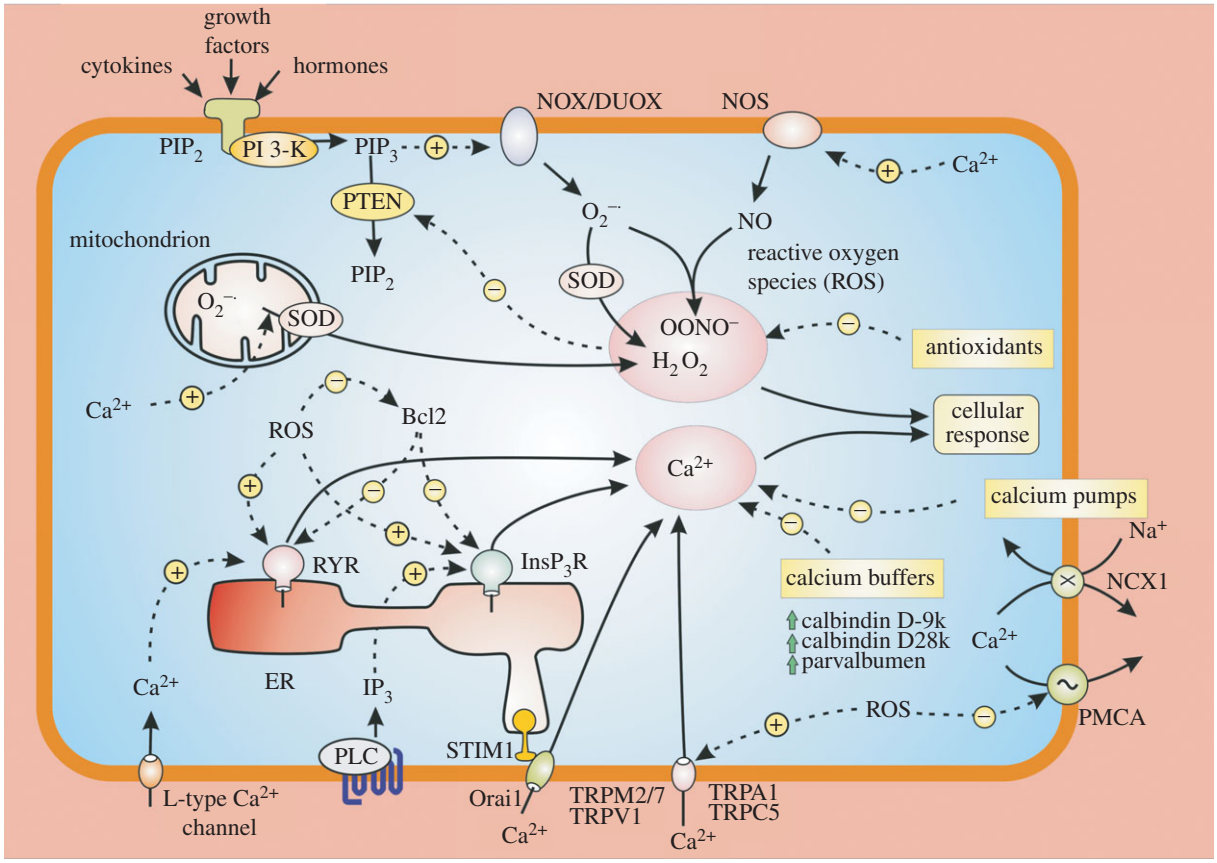

Figure 1. The main reactive oxygen species (ROS) in cells are superoxide ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$). The dashed lines represent the many interactions that operate between the Ca^{2+} and redox signalling pathways. An increase in Ca^{2+} can promote ROS formation by entering the mitochondria to form $O_2^{-\cdot}$ that is converted into H_2O_2 by SOD2. Ca^{2+} can also stimulate the nitric oxide synthase (NOS) that forms NO that interacts with $O_2^{-\cdot}$ to form $ONOO^-$. In a reciprocal way, an increase in cytosolic ROS can markedly enhance Ca^{2+} signalling by either increasing the activity of various channels such as the $InsP_3Rs$ and RYRs or by inhibiting the PMCA pump.

integrity of cell signalling pathways such as those regulated by Ca^{2+} and reactive oxygen species (ROS) [1–3]. It will be argued that low vitamin D levels result in an increase in the activity of these two signalling pathways that not only act to accelerate the ageing process but may also set the stage for the onset of a large number of diseases.

2. Integrated calcium and redox signalling pathways

A large number of cellular processes are regulated by Ca^{2+} signalling pathways often operating in conjunction with the redox signalling pathway [1,2]. What is remarkable about these two signalling systems is the way they interact with each other [4] (figure 1). When Ca^{2+} builds up within the mitochondrion, it increases mitochondrial metabolism resulting in an increased formation of superoxide (O_2^-). Another action of Ca^{2+} is to stimulate nitric oxide synthase (NOS) to increase the formation of NO that contributes to the generation of peroxynitrite ($ONOO^-$). Similarly, ROS can enhance Ca^{2+} signalling. For example, ROS sensitizes both the RYRs [5–7] and the $InsP_3Rs$ [8] to increase the release of Ca^{2+} from the internal store. The expression of Bcl-2, which regulates Ca^{2+} signalling by controlling Ca^{2+} release by the $InsP_3$ receptors [9] and RYRs [10,11], is reduced by ROS [12]. ROS can activate a number of TRP channels that gate Ca^{2+} (e.g. TRPM2, TRPA1 and TRPV1) [13].

The reason for concentrating on these two pathways is because the expression of many of the genes responsible for regulating them is controlled by vitamin D [1,2]. Any deficiency in vitamin D will result in an alteration in how they operate, and this can have profound consequences for many different cellular processes and may be responsible for triggering a number of the diseases that have been linked to vitamin D deficiency.

3. Vitamin D regulation of the Ca^{2+} and redox signalling pathways

The active component of vitamin D is 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$] that is formed by a series of reactions that begin in the skin where sunlight converts 7-dehydrocholesterol to vitamin D_3 (cholecalciferol) (figure 2). The latter is transferred to the liver where a hydroxyl group is added to the C-25 position by a vitamin D-25 hydroxylase (encoded by the *CYP27A1* gene) to form 25-hydroxyvitamin D_3 [25$(OH)D_3$] that is the immediate precursor for active vitamin D. This 25$(OH)D_3$ is carried in the blood to enter multiple cell types where a 25$(OH)D_3$-1α-hydroxylase (encoded by the *CYP27B1* gene) adds another hydroxyl group to the 1 position to form the active hormone 1α,25$(OH)_2D_3$, which will be referred to hereafter as vitamin D, that functions to regulate many different cellular processes [14].

Vitamin D can act in two ways. Firstly, it has non-genomic actions where it alters the activity of various signalling

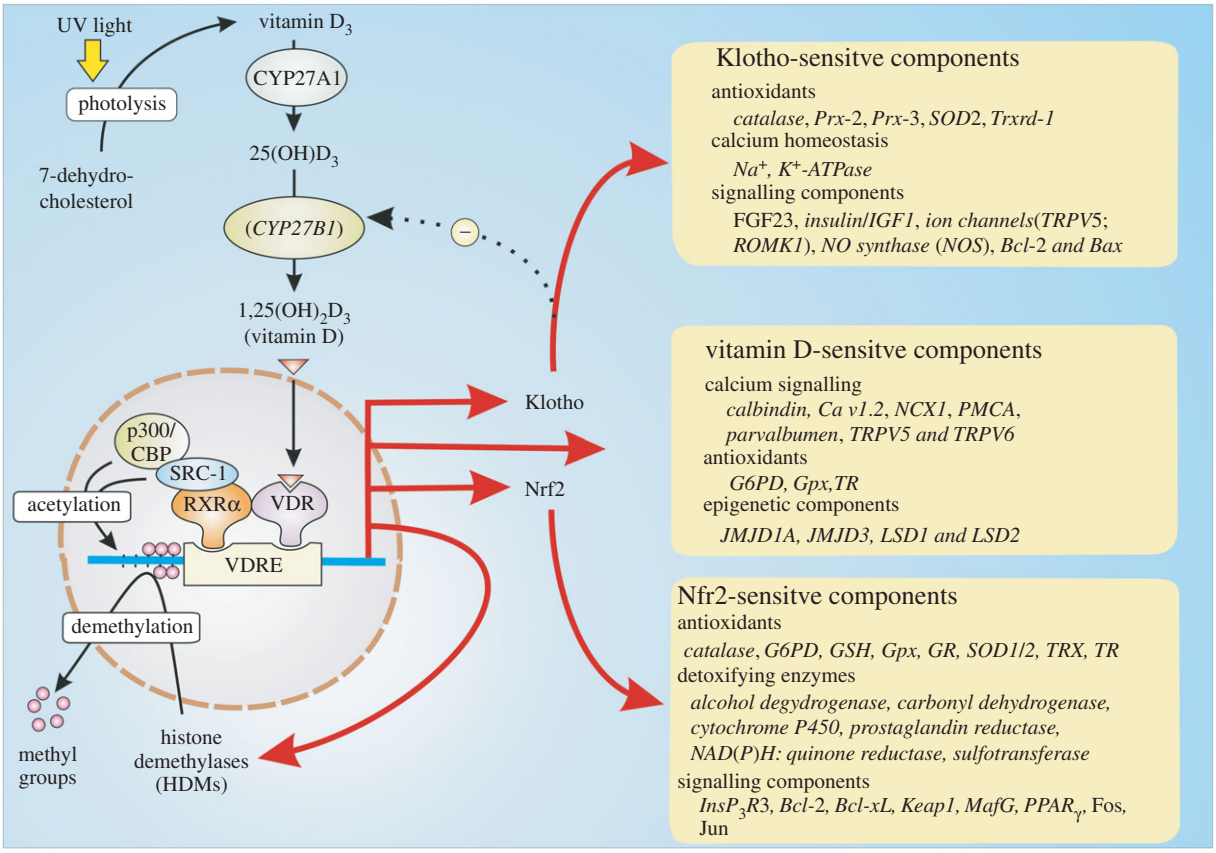

Figure 2. The vitamin D hormone 1,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$] binds to the vitamin D receptor (VDR) that interacts with the retinoid X receptor (RXR) to form the VDR/RXR heterodimer that binds to the vitamin D response element (VDRE). Once in place, the VDR initiates the expression of a large number of genes located in many different cell types to express proteins that function in a number of cellular processes. Many of its actions also depend on its ability to increase the expression of both Klotho and Nrf2 that carry out many of its homeostatic functions.

pathways. Secondly, it has a genomic action that is mediated by its binding to the vitamin D receptor (VDR), which interacts with the retinoid X receptor (RXR) before binding to the vitamin D response element (VDRE) located on a large number of vitamin D-sensitive target genes (figure 2). Two of the important genes that are activated by vitamin D are *Nrf2* and the anti-ageing gene *Klotho*, both of which have multiple roles in maintaining the integrity of cellular signalling systems (figure 2). Many of the genes that are controlled by the vitamin D/Klotho/Nrf2 regulatory network function to maintain Ca^{2+} and redox homeostasis. For example, vitamin D increases the expression of Ca^{2+} pumps, exchangers and buffers to maintain low levels of Ca^{2+}. In addition, vitamin D together with Klotho and Nrf2 all increase cellular antioxidants to maintain the normal reducing environment within the cell [15,16].

Vitamin D may also play a significant role in regulating the balance between autophagy and apoptosis [17,18]. This ability of vitamin D to promote autophagy over apoptosis may depend on its ability to regulate Ca^{2+} signalling that plays a significant role in controlling autophagy [19,20]. Subsequent studies revealed that the $InsP_3/Ca^{2+}$ signalling pathway plays a prominent role in regulating autophagy [21–23]. Elevation in various pathological aggregates such as amyloid, tau, α-synucleins and mutant Huntington fragments, which contribute to neurodegenerative disease such as AD, Parkinson's disease and Huntington's disease, may accumulate because of a decline in autophagy due to an alteration in Ca^{2+} signalling that occurs when vitamin D is deficient.

Another important action of vitamin D is to control the epigenetic landscape of multiple gene promoters to maintain the transcription activity of all the genes that function in its regulatory network [24]. Vitamin D influences the epigenetic landscape by controlling both the acetylation and methylation states of multiple gene promotor regions. The VDR/RXR dimer recruits histone acetyltransferases (HATs) such as p300/CBP and steroid receptor coactivators 1 and 2 (SRC1 and SRC2) that carry out the acetylation reactions that open up the chromatin structure to facilitate transcription so as to maintain phenotypic stability (figure 2).

Vitamin D can also regulate phenotypic stability by regulating demethylation. Many of the genes regulated by vitamin D are silenced by methylation of CpG islands located in their promotor regions [25]. For example, the decline in SERCA2a activity in cardiovascular disease may be caused by hypermethylation of its promotor region [26]. Expression of the *Klotho* gene, which acts together with vitamin D to regulate phenotypic stability, is silenced by methylation [27,28]. Such hypermethylation of promotor regions increases during ageing and is evident in many of the diseases such as cancer, cardiovascular and neurodegenerative diseases [29]. For example, hypermethylation of promotors in GABAergic neurons may contribute to the phenotypic remodelling responsible for schizophrenia and bipolar disorder [30]. Vitamin D modulates methylation by inducing the expression of a number of key DNA demethylases such as Jumonji domain-containing protein 1A and 3 (JMJD1A, JMJD3) and lysine-specific demethylase 1 and 2 (LSD1, LSD2) that contributes to its ability to maintain phenotypic stability [31].

This ability of vitamin D to modulate the epigenetic landscape is in keeping with its proposed role in maintaining

phenotypic stability, and this may explain why vitamin D deficiency has been linked to both ageing and so many of the age-related diseases.

4. Vitamin D and ageing

There is increasing evidence that vitamin D may play an important role in the process of ageing. For example, the decline in cognition that occurs normally in older adults has been linked to vitamin D deficiency [32–35]. The ability of human skin to synthesize vitamin D declines with age [36], and this may account for the decline in the level of vitamin D and Klotho during ageing. Vitamin D and Klotho deficiency may contribute to the ageing process through dysregulation of the Ca^{2+} and redox cell signalling pathways. Nrf2 may also act to regulate longevity [37]. Dysregulation of Ca^{2+} signalling, which is closely linked to mitochondrial dysfunction and ROS formation, has been implicated in ageing [38,39]. In ageing striatal neurons, there is a marked decline in the expression of Bcl2 [38], which would contribute to the dysregulation of Ca^{2+}, because one of its functions is to inhibit the $InsP_3Rs$ [9] (figure 1).

There has long been an interest in the possibility that alterations in the cellular redox balance [40,41] and Ca^{2+} signalling [42] might be responsible for ageing [43]. The way in which vitamin D deficiency and a concomitant decline in both Klotho and Nrf2 function contributes to many diseases may be explained through the ability of these custodial systems to maintain the stability of the redox and Ca^{2+} signalling systems described earlier [2]. For example, during ageing, there is a decline in the capacity of cells to maintain NAD(P)H levels in neurons [44,45], and this accounts for a decline in the levels of glutathione (GSH), which is essential to maintain low redox levels [46]. Such a decline in GSH results in a selective decline in the activity of GABAergic neurons in the hippocampus and could contribute to schizophrenia [47]. Vitamin D acts to maintain the expression of the Nrf2 antioxidant pathway [48]. There is a marked decline in the level of Nrf2 in the AD brain compared with age-matched controls [49]. Genetic ablation of the VDR results in premature ageing in mice suggesting that vitamin D can maintain normal physiological ageing [50].

Some of the most convincing evidence that vitamin D deficiency contributes to the ageing process has emerged from studies on the decline of memory in ageing rats. When considering memory mechanisms, it is important to point out that the ageing process does not affect long-term memories, but it induces a slow and progressive deterioration in the formation and retention of new memories [51]. This initial age-related decline in working memory is very subtle and has been linked to small changes in both the Ca^{2+} and redox signalling pathways [51–53]. An alteration in Ca^{2+} signalling has been linked to ageing in the brain [54–58]. The early loss of memory is caused by a number of subtle changes such as an elevation in the resting level of Ca^{2+} [56] and an increase in the expression of the $Ca_V1.2$ L-type Ca^{2+} channel [58], which is one of the proteins that is normally down-regulated by vitamin D (figure 2). Such changes may also depend on a decrease in the neuronal Ca^{2+} buffers and a decline in the mechanisms responsible for extruding Ca^{2+} from the cytoplasm [59]. Enhancing the intracellular buffering capacity markedly enhanced the learning capacity of aged rats [60].

At the electrophysiological level, the loss of memory during ageing has been linked to the progressive increase in the amplitude of the slow after hyperpolarization (sAHP) [52,61]. This sAHP is caused by increased fluxes of Ca^{2+} through $Ca_V1.2$ L-type voltage-gated channels, which are known to be elevated during ageing [58], and the RYRs resulting in abnormally high Ca^{2+} transients that activate SK potassium channels to hyperpolarize the membrane [51,55,57]. This sAHP reduces working memory in two ways. Firstly, the hyperpolarization reduces the spiking activity necessary for memory formation through long-term potentiation (LTP). Secondly, the increase in Ca^{2+} stimulates calcineurin to induce the long-term depolarization (LTD) that erases memories [52].

One of the interesting aspects of this dysregulation is that the relatively subtle elevation in the sensitivity of the Ca^{2+} signalling pathway appears to be driven by an increase in the oxidative state of the neurons [62]. In ageing mice, there is a marked increase in oxidative stress that contributes to a reduction in memory formation [63]. The enhanced ROS levels may increase sAHP by sensitizing the RYRs (figure 1). This would seem to be the case because the sAHP can be reversed by treating neurons with dithiothreitol (DTT) [64]. Similarly, a decrease in ROS could also contribute to the increase in cognition observed in ageing rats following treatment with the anti-inflammatory drug montelukast that is used normally to treat asthma [65]. The dysregulation of both Ca^{2+} and ROS signalling that is responsible for development of the sAHP during ageing seems to depend on vitamin D deficiency. The vitamin D/Klotho/NRF2 regulatory system can prevent the dysregulation of the Ca^{2+} and ROS signalling responsible for the sAHP through multiple mechanisms. For example, vitamin D suppresses the expression of the $Ca_V1.2$ L-type Ca^{2+} channel [66] that initiates the Ca^{2+} signal that induces the sAHP, and it also maintains the expression of PMCA and NCX1, which extrude Ca^{2+} from the cell. Klotho acts to stimulate the Na^+/K^+-ATPase responsible for maintaining the Na^+ gradient necessary for Ca^{2+} extrusion by NCX1. Finally, NRF2 increases the expression of many antioxidants that ensure that ROS levels are kept low, which will prevent the sensitization of the RYRs that are triggering the sAHP and memory erasure.

Such a conclusion is strongly supported by the observation that vitamin D can reverse the Ca^{2+}-dependent processes responsible for the age-related decline in memory [67]. What is more significant is that vitamin D can enhance hippocampal synaptic function, and more significantly, it could prevent the decline in cognition [68].

The fact that vitamin D deficiency brings about a dysregulation of both the Ca^{2+} and redox signalling pathways during ageing has raised an interesting possibility that it could also contribute to the initiation of age-related diseases [2].

5. The vitamin D/Klotho/Nrf2 regulatory network and disease

While most attention has been focused on establishing a link between vitamin D deficiency and disease, there is little information as to what the mechanism might be. To answer this question, I have developed a phenotypic stability hypothesis that is based on the idea that vitamin D may play an essential role through its ability to maintain both the redox and Ca^{2+}

signalling pathways as described earlier [1,2]. A decline in the activity of the vitamin D/klotho/Nrf2 regulatory network has been linked to many diseases. Roselli & Caroni [69] have emphasized the importance of studying the early preclinical phases of neurodegenerative diseases. AD is a case in point in that the preclinical phase can last for many years before the disease is diagnosed. The following conceptual framework attempts to explain what might drive the early preclinical disease development and how this may be related to the ageing process. The basic idea is that there is a slow but progressive dysregulation of the Ca^{2+} and redox signalling pathways resulting from a deficiency in vitamin D [1,2]. It will be argued that this dysregulation results in an alteration in the modulatory activity of the $InsP_3/Ca^{2+}$ signalling pathway, and this creates subtle alterations in the normal cellular signalling pathway resulting in the onset of disease. To understand why such subtle alterations occur can lead to various disease states, it is important to consider the way the Ca^{2+} signalling system is organized in each specific cell type to provide either primary or modulatory signals.

(a) Cardiovascular disease

Vitamin D deficiency has been linked to hypertension and cardiovascular disease [70–76]. The ability of vitamin D to protect the cardiovascular system may depend on its ability to maintain the stability of the ROS and Ca^{2+} signalling systems, which are known to be dysregulated in hypertension, cardiac hypertrophy, congestive heart failure (CHF) and atrial arrhythmias.

One of the main causes of cardiac hypertrophy and CHF is hypertension. The renin–angiotensin system (RAS) plays a major role in regulating blood pressure. One of the primary actions of vitamin D is to curb RAS to prevent the hypertension that is a major risk factor for heart disease [77]. Vitamin D regulates the secretion of renin by renin-producing granular cells, which is controlled by the cyclic AMP signalling pathway. Vitamin D acts by preventing the cyclic AMP response element-binding protein (CREB) from binding to the renin gene promotor [78]. In mice, deletion of either the enzyme 25(OH)D 1α-hydroxylase or the VDR resulted in an increase in the renin–angiotensin system, hypertension and the onset of cardiac hypertrophy [79–81]

In patients with type 2 diabetes, the associated hypertension was improved following vitamin D supplementation [82]. The excessive release of renin and the resulting increase in angiotensin II can have multiple effects on some of the key components of the cardiovascular system. One of the actions of angiotensin II is to increase the formation of endothelin-1 (ET-1), which is a potent vasoconstrictor and thus contributes to angiotensin II-induced hypertension [83,84].

The changes in Ca^{2+} signalling in ventricular cardiac cells, which result in hypertrophy and CHF, are relatively minor. There is a small increase in the amplitude of the Ca^{2+} transient that occurs during each heartbeat. This amplification of each transient is caused by an increase in the activity of $InsP_3/Ca^{2+}$ modulatory signalling pathway, which is driven by the increased levels of angiotensin II and ET-1 [85]. In the presence of these two hormones, there are subtle changes in the spatial properties of the individual Ca^{2+} transients. It was proposed that the increase in $InsP_3$ acts on perinuclear $InsP_3R2$s to create a nuclear Ca^{2+} signal responsible for driving the transcriptional processes that initiate hypertrophy [86] (figure 3). There is now considerable experimental evidence to show that activation of $InsP_3Rs$ can indeed function to induce the nuclear Ca^{2+} transients that activate the transcriptional events responsible for the onset of hypertrophy [87–93]. One of the genes that is activated is *ITPR2* that codes for the $InsP_3R2$ that is responsible for the nuclear Ca^{2+} signal that drives hypertrophy [94]. In cardiomyoctes, miR-133a acts to inhibit the expression of $InsP_3R2$ [95]. Down-regulation of miR-133a accounts for an increase in the level of the $InsP_3R2$s, and this is a major contributory factor for the onset of cardiac hypertrophy.

Vitamin D deficiency contributes to the onset of hypertrophy by increasing the Ca^{2+} and redox signalling pathways. For example, there is a decrease in the expression of both SERCA and phospholamban (PLN) that contributes to an increased Ca^{2+} transient amplitude and a decline in the recovery phase [96]. Vitamin D deficiency will also result in an increase in ROS levels that then enhances the Ca^{2+} signalling events that initiate the processes of hypertrophy that results in CHF [97,98]. The angiotensin II and ET-1 not only act to increase Ca^{2+}, but they also increase ROS levels by stimulating NOX at the plasma membrane [99–101] (figure 3). ROS acts by increasing the activity of the ion channels ($Na_V1.5$ sodium channel, $Ca_V1.2$ channels and RYR2) and pumps (SERCA) that contribute to the Ca^{2+}cycling events that occur during each heartbeat. In addition, ROS can also act indirectly by increasing the activity of protein kinases such as PKA and $CaMKII\delta_c$ that act normally to regulate cardiac activity [101]. These increased ROS and Ca^{2+} signalling processes contribute to the alterations in gene transcription that result in hypertrophy [98]. The cardiac hypertrophy in spontaneously hypertensive rats is reduced by vitamin D [102], and vitamin D supplementation can also markedly improve the outcome of patients suffering from heart failure [74,103].

(b) Alzheimer's disease

AD is another example of a major human disease where the initial change is so subtle that it can go undiagnosed for long periods. The initial symptoms are a decline in working memory, which closely resemble those that occur in ageing as described earlier. The onset of AD depends on the accumulation of extracellular β-amyloid (Aβ) deposits that disrupt neuronal signalling pathways to reduce cognition. The Ca^{2+} hypothesis considers that the loss of memory depends on an up-regulation of neuronal Ca^{2+} signalling [104–109]. When Ca^{2+} is measured in the spines and dendrites of cortical pyramidal neurons of transgenic mice, there was a higher than normal resting level in those neurons located close to amyloid deposits [110]. Similarly, the resting level of Ca^{2+} in the cortical neurons of 3xTg-AD animals was 247 nmol l^{-1}, which was twice that found in the non-Tg controls (110 nmol l^{-1}) [111]. Such evidence of a persistent elevation in the resting level of Ca^{2+} led to the suggestion that it may continuously activate LTD to explain why memories are erased shortly after they are formed [112,113].

The relatively small elevation in the resting level of Ca^{2+} does not alter the overall function of the brain. Information from the sensory organs can still be processed, new memories can be formed, but they are not retained because the persistent elevation in Ca^{2+} erases them shortly after they are formed. A number of mechanisms have been proposed to explain the

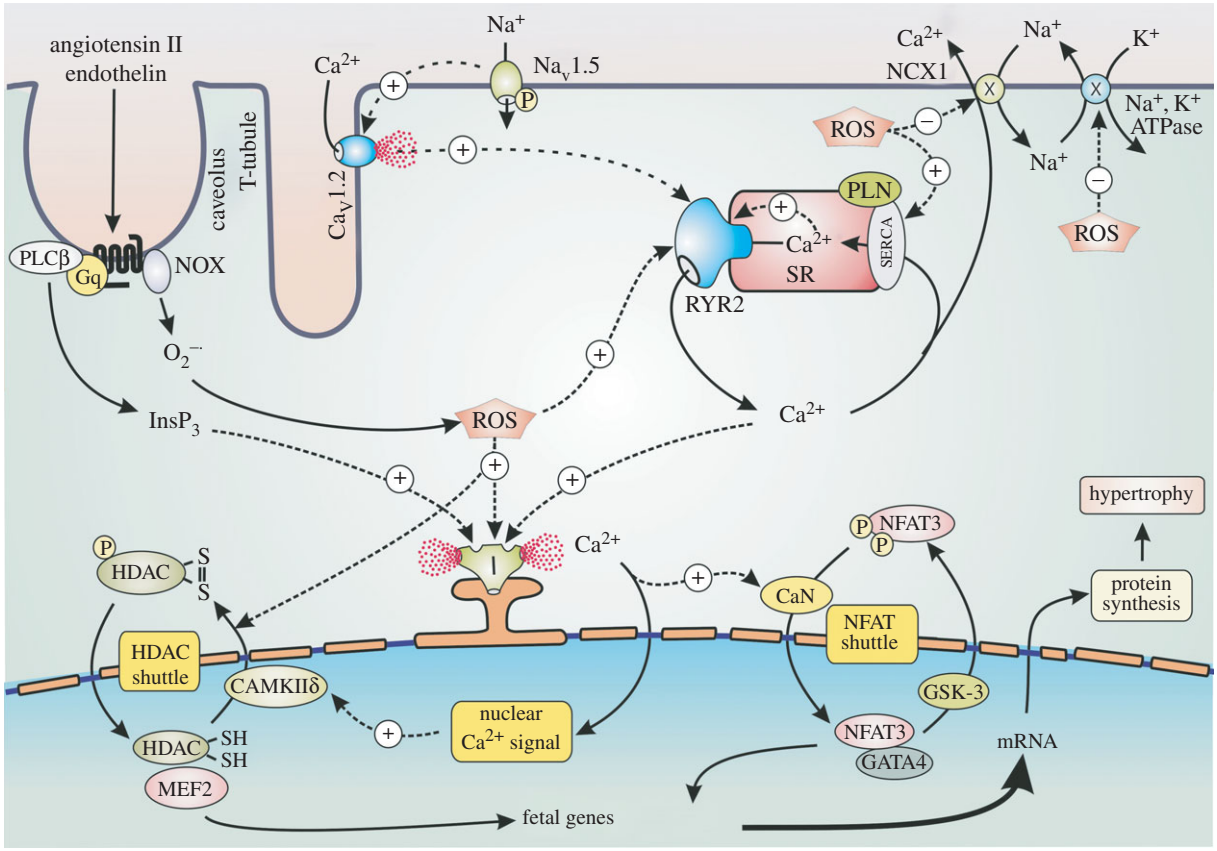

Figure 3. The role of enhanced Ca^{2+} and ROS levels in cardiac hypertrophy. A number of signalling pathways have been implicated in the activation of hypertrophy. A major pathway is induced by angiotensin II and endothelin that stimulate the formation of $InsP_3$ that triggers a nuclear Ca^{2+} signal that activates the HDAC and NFAT shuttles to stimulate the transcription factors responsible for switching on the fetal genes that induce hypertrophy. These hormones also activate NOX to form reactive oxygen species (ROS) that contributes to hypertrophy by enhancing the sensitivity of $InsP_3R$ (I) and ryanodine receptor 2 (RYR2).

elevation of intracellular Ca^{2+} levels by the Aβ protein [114,115]. Many of these mechanisms depend on the $InsP_3$/Ca^{2+} signalling pathway [116–119]. Aβ can bind to the cellular prion protein (PrP^C), which is coupled to mGluR5 that increases $InsP_3$ formation and Ca^{2+} release [117] (figure 4). The formation of $InsP_3$ is also increased by Aβ acting on the calcium-sensing receptor (CaSR) [118]. Phospholipase Cη1 (PLCη1), which is activated by Ca^{2+}, may contribute to the dysregulation of Ca^{2+} signalling by amplifying these Aβ-dependent elevations in Ca^{2+} [119]. Activation of the mGluR5 receptor by the Aβ protein has been shown to enhance the process of LTD responsible for memory loss [120]. The significance of $InsP_3R$ activation in the pathogenesis of AD has also emerged from studies on the effects of presenilin mutations. In familial Alzheimer's disease (FAD), presenilin mutations enhance the activity of $InsP_3Rs$ resulting in an increase in Ca^{2+} signalling in both human cells and mouse neurons [121,122]. In a mouse model of AD, which had mutations in presenilin, the AD symptoms were reversed following a reduction in the expression of the $InsP_3R$, thus supporting the notion that the $InsP_3$/Ca^{2+} signalling pathway plays a significant role in disease pathogenesis [122].

There are an increasing number of studies indicating that a deficiency in vitamin D may contribute to the onset of AD [123–128]. The level of vitamin D in AD patients is lower than that in controls [129]. Enhanced dietary vitamin D intake lowered the risk of developing AD in a study of older women [130]. VDR polymorphisms have been associated with age-related decline in cognition and are also a risk factor for AD [126,131,132]. Since AD seems to be caused by abnormal elevations in Ca^{2+}, it is reasonable to propose that the deleterious effect of vitamin D deficiency may be explained by a decrease in its normal role as a custodian of Ca^{2+} and ROS homeostasis. Similarly, a decrease in ROS could also contribute to the increase in cognition observed in ageing rats following treatment with the anti-inflammatory drug montelukast that is used normally to treat asthma [65].

Vitamin D may prevent the onset of AD by regulating a number of processes. Firstly, vitamin D can increase the expression of the multidrug resistance protein 1 (*MDR1*) gene that codes for the P-glycoprotein (P-gp), which is an efflux transporter that acts to reduce the accumulation of Aβ [133]. Secondly, vitamin D may act to control the expression of those toolkit components responsible for maintaining low ROS and Ca^{2+} levels. For example, vitamin D stimulates the expression of Ca^{2+} pumps and exchangers (PMCA and NCX) and Ca^{2+} buffers such as calbindin (CB) and parvalbumin (figure 4). The expression of neuronal CB is known to be reduced in AD [134]. Mice expressing mutant APP also display a decline in the level of CB especially in the dentate gyrus region of the hippocampus, which functions in learning and memory [135]. Vitamin D can curb the influx of external Ca^{2+} by reducing the expression of L-type voltage-sensitive channels, which are markedly elevated in rat hippocampal neurons [66].

Many of the deleterious effects of vitamin D deficiency in AD may depend on a decline in the expression of

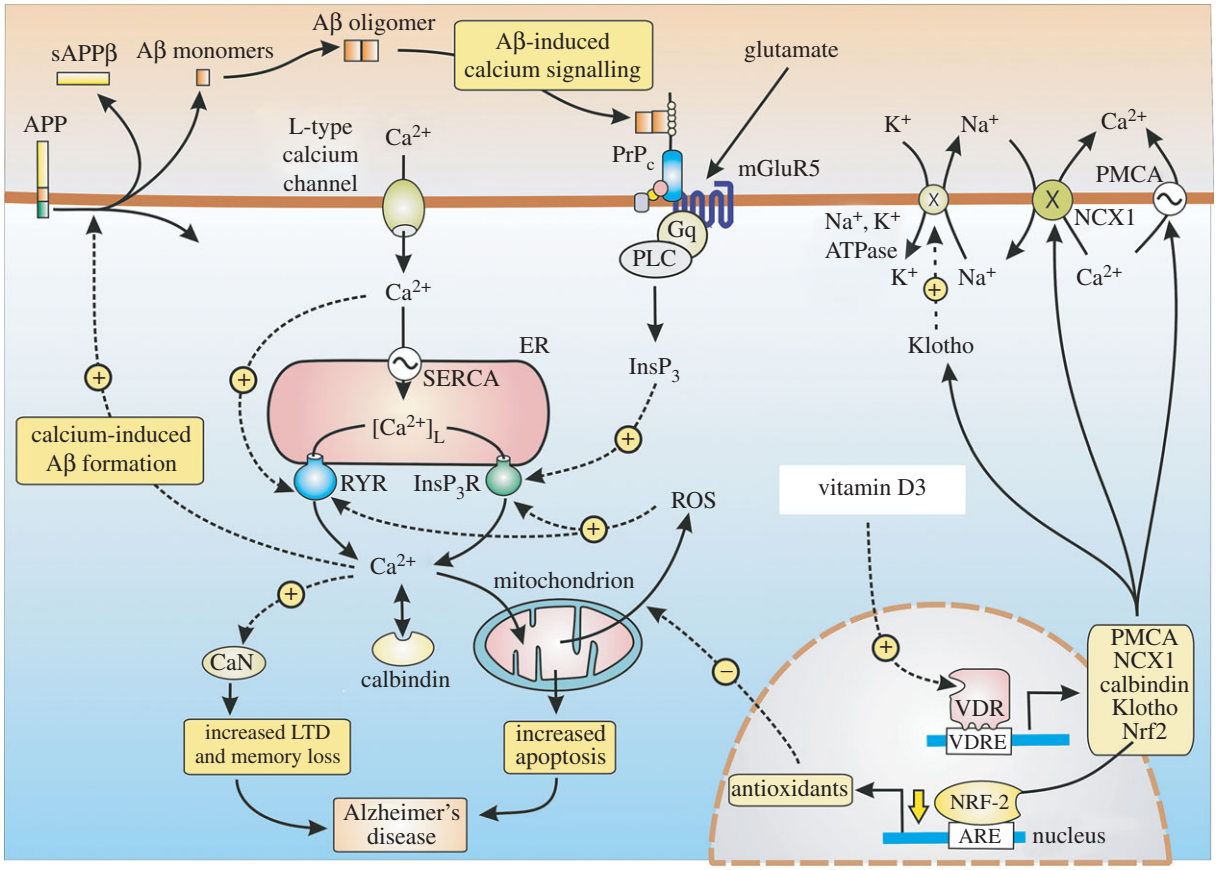

Figure 4. Dysregulation of Ca^{2+} and redox signalling in Alzheimer's disease (AD). The calcium hypothesis of AD suggests that the formation of Aβ oligomers brings about an overall increase in Ca^{2+} signalling that results in a permanent elevation in the resting level of Ca^{2+} to 300 nM that then erases memories soon after they are formed by activating calcineurin (CaN) inducing long-term depression (LTD). An elevation of Ca^{2+} sets up a positive feedback loop by acting to stimulate the hydrolysis of the amyloid precursor protein (APP) to generate the Aβ oligomers that bind to the cellular prion protein (PrP^C) that then activates the $InsP_3/Ca^{2+}$ signalling pathway. Vitamin D reduces the risk of AD by acting to maintain Ca^{2+} and redox signalling at their normal low resting levels.

its two collaborators Nrf2 and klotho. Nrf2 levels are markedly reduced in the brain of patient with AD [49]. Vector-mediated expression of Nrf2 in the hippocampus of AD transgenic mice resulted in a marked improvement in cognition [135]. One of the main functions of Nrf2 is to maintain the cellular level of the redox buffer GSH [136], which is a critical factor in preventing AD [44]. The level of Bcl-2, which inhibits the ability of $InsP_3$ to activate the $InsP_3$ receptors [9] and the RYRs [10,11] (figure 1), is maintained by Nrf2 and Klotho, thereby reducing the level of Ca^{2+}. The ability of Bcl-2 to reduce the symptoms of AD in transgenic mice [137,138] may be explained by this reduction in the activity of both the $InsP_3R$ and RYRs. Klotho may also play a role in AD, because its levels in the CSF of patients with AD are lower than those in age-matched controls [139]. In the senescence-accelerated mouse prone-8 (SAMP8) mouse, a decline in the expression of klotho has been linked to symptoms of AD, including a decline in cognition and an accumulation of amyloid-β_{1-42} [140].

It is clear that dysregulation of the vitamin D/klotho/Nrf2 regulatory network results in a decline in cell signalling stability that results in the elevated neuronal Ca^{2+} and ROS levels that seem to responsible for the onset of AD. Such a mechanism suggests an interesting explanation for the sporadic nature of AD. Despite it being referred to as an age-related disease, not everyone who ages develops AD. So what is it that triggers the onset of sporadic AD? One possibility is that it is induced in those individuals who are deficient in vitamin D and thus have abnormally elevated levels of Ca^{2+} that may initiate the formation of the pathological Aβ oligomers [2,3]. This possibility is supported by the fact that Ca^{2+} acts to stimulate the formation of Aβ (figure 4) [57,107,141–145]. Inhibiting the RYR2 with dantrolene that reduces their release of Ca^{2+} was found to markedly reduce the formation of Aβ [145]. Such Ca^{2+}-induced increases in amyloid formation then initiates a positive feedback loop, because it is followed by Aβ-induced Ca^{2+} signalling and it is this $A\beta/Ca^{2+}$ positive feedback loop that may be responsible for the onset of AD [146]. Such a scenario is entirely consistent with the fact the vitamin D deficiency is such a strong risk factor for AD.

6. Conclusion

The phenotypic stability of the interacting Ca^{2+} and ROS signalling pathways is maintained by vitamin D. It is argued that a deficiency in vitamin D results in an elevation in both the ROS and Ca^{2+} signalling pathways that may contribute to the process of ageing. An example of this is the age-related decline in the cognition of rats that can be reversed by administering vitamin D. Such deficiencies in vitamin D may also set the stage for the onset of both heart disease and AD.

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