

Rapid non-genomic actions of 25(OH)D₃: state of the art

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ABSTRACT

Over recent years, the secosteroid hormone calcitriol (1 α ,25(OH)₂D₃) has been attracting growing attention due to its essential role in calcium absorption and bone mineralization. This hormone elicits these functions through genomic and non-genomic mechanisms. In the former case, the interaction of 1 α ,25(OH)₂D₃ with vitamin D receptor (VDR) results in the transcription of genes involved in the regulation of calcium homeostasis. Compared with their genomic counterparts, non-transcriptional effects, on the other hand, occur rapidly and are not subject to the effects of transcription and protein synthesis inhibitors; they have also been shown to be responsible for the multiple actions of vitamin D. The direct precursor metabolite of 1 α ,25(OH)₂D₃, calcifediol (25(OH)D₃), which also exhibits anti-proliferative and gene regulatory properties, was recently described as an agonistic ligand of VDR, albeit with lower affinity than 1 α ,25(OH)₂D₃. This mini-review attempts to offer an overview of the non-genomic actions of calcifediol and the possible mechanisms underlying the generation of these rapid responses. Insights into the rapid non-genomic mechanisms of 25(OH)D₃ could help to increase knowledge of the vitamin D endocrine system, and thus result in the identification of novel therapeutic strategies able to regulate non-genomic actions, which could prove crucial in 25(OH)D₃ deficiency-related disorders.

KEYWORDS

Vitamin D3, calcifediol, rapid non-genomic effects, vitamin D receptor, membrane-associated rapid response to steroid.

1 α ,25(OH)₂D₃ and rapid non-genomic actions

Calcitriol (1 α ,25(OH)₂D₃) is a secosteroid hormone synthesized in the skin with known biological functions. It is involved in the maintenance of bone and calcium/phosphorous homeostasis^[1]. Its biological effects are classically known to be mediated via interaction with a nuclear vitamin D receptor, which contains an N-terminal DNA-binding domain, and a conserved C-terminal ligand-binding domain (LBD). Upon interaction of 1 α ,25(OH)₂D₃ with the LBD, the vitamin D receptor (VDR) undergoes a structural change that results in co-repressor dissociation from the receptor and its association with the retinoid X receptor (RXR) via the dimerization domains. The resulting 1 α ,25(OH)₂D₃–VDR–RXR complex binds to the vitamin D-responsive elements, repeated sequences positioned upstream of the start site of the target genes encoding proteins involved in the regulation of calcium homeostasis, thereby promoting or repressing their transcription^[1–3].

It is recognized that 1 α ,25(OH)₂D₃ also exerts rapid non-genomic actions that are responsible for the various actions of vitamin D.

Non-genomic actions were first described in Hans Selye's pioneering work in 1942. He discovered that progesterone, injected into rats, immediately produces rapid and deep anaesthesia, in contrasting to what was observed with the main action of the hormone, which took place within hours or days after its

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application^[4]. Decades later, *in vitro* studies carried out on dog erythrocytes by Spach and Streeten revealed that exchange of Na⁺ ions occurred within minutes of aldosterone administration. They thus offered new evidence of a non-genomic effect of steroids in cells lacking a nucleus, in which the possibility of transcription and therefore of genomic action is excluded^[5].

Although other rapid non-genomic effects were subsequently established, they were not clarified until their recent identification for several steroid hormones, including 1 α ,25(OH)₂D₃^[6].

The peculiarity of a rapid non-genomic response is that it occurs rapidly (within seconds to minutes, is not blunted by inhibitors of transcription and protein synthesis, such as actinomycin D and cycloheximide, and takes place also in response to steroids bound to large macromolecules which hamper their cell entering^[7]. In summary, the main criteria for defining actions non-genomic as opposed to genomic are the sensitivity of transcription and protein synthesis inhibitors and the time course.

Membrane-associated proteins that mediate rapid non-genomic actions of $1\alpha,25(\text{OH})_2\text{D}_3$

With regard to earlier observations of an independent mechanism of genome transcription and subsequent protein synthesis induced by $1\alpha,25(\text{OH})_2\text{D}_3$, Nemere *et al.* [81], in 1984, observed that $1\alpha,25(\text{OH})_2\text{D}_3$ induced a rapid (within 14 min) unidirectional increase in Ca^{2+} transport from the lumen to the venous effluent in normal, vitamin D-replete chicks.

Over the last years, rapid non-genomic responses have attracted as much interest as genomic actions, and considerable efforts have been made to address how these rapid effects are mediated, and therefore what receptors are involved.

Some studies suggest that the non-transcriptional effects begin at the plasma membrane and could involve a distinct membrane VDR (mVDR), as described by Norman *et al.* [9,10], or a non-classical, membrane-associated, rapid response $1,25(\text{OH})_2\text{D}_3$ -binding receptor called $1,25(\text{OH})_2\text{D}_3$ -MARRS. Binding of $1,25(\text{OH})_2\text{D}_3$ at the cell membrane could stimulate several signalling molecules, including phospholipase A2 (PLA2), phosphatidylinositol-3 kinase (PI3K), and phospholipase C, resulting in the generation of one or more second messengers, such as Ca^{2+} ions, phosphatidylinositol (3,4,5)-trisphosphate, and cyclic AMP, thereby activating different downstream protein kinases (calcium/calmodulin-dependent protein kinase II gamma, protein kinase C [PKC], SRC proto-oncogene, non-receptor tyrosine kinase [Src], and mitogen-activated protein kinases) [11-15].

Additional evidence shows that mVDR could bind to proto-oncogene Src and caveolin 1 in caveolae-enriched plasmalemma, thereby acting as a regulator of multiple signalling pathways, such as the Wnt [16-19], sonic hedgehog [20-25], and Notch [26-28] ones.

The best-described MARRS that binds $1,25(\text{OH})_2\text{D}_3$ is the protein disulphide isomerase family A, member 3 (Pdia3) [29]. Its main function is to catalyse the rearrangement of non-native disulphide bonds, interacting with chaperone proteins, calnexin and calreticulin, to mediate the correct folding of nascent glycoproteins [30]. One remarkable function of Pdia3 is its participation in one of the most evident rapid non-transcriptional actions of $1\alpha,25(\text{OH})_2\text{D}_3$, called transcaltachia [12,31,32]. Furthermore, the interaction of $1,25(\text{OH})_2\text{D}_3$ with Pdia3 has been reported to have significant repercussions on protection against UV-induced pyrimidine dimerization [33], the repression of tumor necrosis factor receptor signalling pathway induced by a rapid increase in intracellular Ca^{2+} concentration in aortic smooth muscle cells [34], and stimulation of PKC signalling cascades [35].

$25(\text{OH})\text{D}_3$ -related rapid non-genomic actions

Calcifediol has long been recognised as a prohormone activated by the 1α -hydroxylase activity in the proximal tubule of the kidney. It is the major circulating metabolite of vitamin D. It has a half-life of approximately three weeks, and its highest blood concentration is used as the best indicator of total vitamin D storage. This vitamin D metabolite is produced by the hydroxylation at the C-25 position of vitamin D_3 , which is

produced in the skin from 7-dehydrocholesterol (7-DHC) in the presence of ultraviolet light.

Recent studies have suggested that $25(\text{OH})\text{D}_3$ can regulate gene expression through direct binding to VDR [36-40]. The findings of a study by Lou *et al.* [36] suggested that $25(\text{OH})\text{D}_3$ is an agonist VDR ligand with anti-proliferative effects and gene regulatory properties. These authors found a larger ligand-binding pocket volume for $25(\text{OH})\text{D}_3$ than for $1,25(\text{OH})_2\text{D}_3$. This, along with the absence of two additional 1α -hydroxylase-generated hydrogen bonds, explains why the affinity of $25(\text{OH})\text{D}_3$ for VDR is lower than that of the biologically active metabolite of vitamin D_3 . In detail, it has been reported that VDR binds to $25(\text{OH})\text{D}_3$ with an affinity 50 times lower than it does to $1,25(\text{OH})_2\text{D}_3$. In the same study, additional experiments performed on primary mouse cells derived from skin, kidney and prostate cells, the MCF-7 breast cancer cell line, and the LN-CaP prostate cancer cell line, demonstrated that $25(\text{OH})\text{D}_3$ possesses gene regulatory functions and anti-proliferative effects that are mediated by the nuclear VDR. Similar results were obtained by Munetsuna *et al.* [41], who demonstrated that $25(\text{OH})$ -19-nor- D_3 inhibited human prostate cell growth in a VDR-dependent and 1α -hydroxylation-independent mechanism.

In view of the abovementioned studies, we assumed, in recent research [42], that $25(\text{OH})\text{D}_3$ could mediate rapid non-genomic actions upon binding to membrane receptors, such as the rapid increase in intracellular Ca^{2+} concentrations observed for the biologically active metabolite of vitamin D. We evaluated this mechanism in mesenchymal stem cells derived from human adipose tissue (hADMSCs), previously described as excellent cell model systems for studying the hormonal effects of $1\alpha,25(\text{OH})_2\text{D}_3$. For the first time, we reported that $25(\text{OH})\text{D}_3$ causes a significant increase in intracellular Ca^{2+} levels in hADMSCs at higher concentrations than are normally present in the body (nanomolar range). The fact that this effect was found at higher-than-physiological levels could be due to the reduced binding affinity of $25(\text{OH})\text{D}_3$ for VDR compared with the active form of vitamin D [36].

This observation was in line with the rapid non-genomic effects of $25(\text{OH})\text{D}_3$ in human spermatozoa, where it has been reported that $25(\text{OH})\text{D}_3$ was unable to influence the Ca^{2+} levels in the subnanomolar range, while a marked but delayed rise of intracellular Ca^{2+} concentration was found in human spermatozoa after stimulation with higher concentrations [43]. In addition to this, the delay in the Ca^{2+} response could be explained by certain metabolic effects due to the presence of 1α -hydroxylase in the neck region of human sperm.

A study by Asano *et al.* [44] revealed a VDR-independent non-genomic effect of $25(\text{OH})\text{D}_3$ on the processing and degradation of sterol regulatory element-binding protein (SREBP) cleavage-activating protein (SCAP) in the endoplasmic reticulum, serving to regulate lipid metabolism. In view of a reported inverse correlation between metabolic syndrome severity and serum $25(\text{OH})\text{D}_3$ levels, they evaluated whether SREBPs might be inhibited by secosteroids. Among the chemical substances investigated, $25(\text{OH})\text{D}_3$, independently of VDR, induced ubiquitin-mediated proteasomal degradation of SREBP/SCAP through a non-genomic mechanism, which resulted in decreased expression of SREBP-responsive genes.

Disruption of endothelial stability and enhancement of vascular leaks have been shown to be involved in infectious diseases, which are prevented by correct supplementation of vitamin D and its metabolites. Data suggested that vitamin D₃-related non-genomic actions play a critical role in mediating both epithelial and endothelial cell stability through mechanisms independent of the canonical transcription-mediated vitamin D pathway [45]. Consequently, vitamin D deficiency could impair the protective epithelial barrier, leading to vascular fluid leakage and worsening infections, thereby causing septicaemia [45]. Therefore, the vitamin D-mediated rapid non-transcriptional functions could facilitate resolution of inflammation and infections, and ensure endothelial junction integrity. As a result, hypovitaminosis D could increase susceptibility to and the gravity of infections and chronic diseases, contributing to a high incidence rate of complications and premature death [46].

The ability of vitamin D to elicit rapid non-genomic effects has also been reported in renal tissues obtained from patients with kidney failure. In addition to current standard replacement therapy with 1,25(OH)₂D₃, intake of cholecalciferol and/or 25(OH)D₃ has been demonstrated to improve quality of life and survival of individuals with chronic kidney disease [47].

Discussion and main conclusions

Over recent years, the secosteroid 1,25(OH)₂D₃ has been demonstrated to regulate many biological processes, including calcium and phosphate homeostasis, cell proliferation, cell differentiation, and cancer invasion and angiogenesis [48-51].

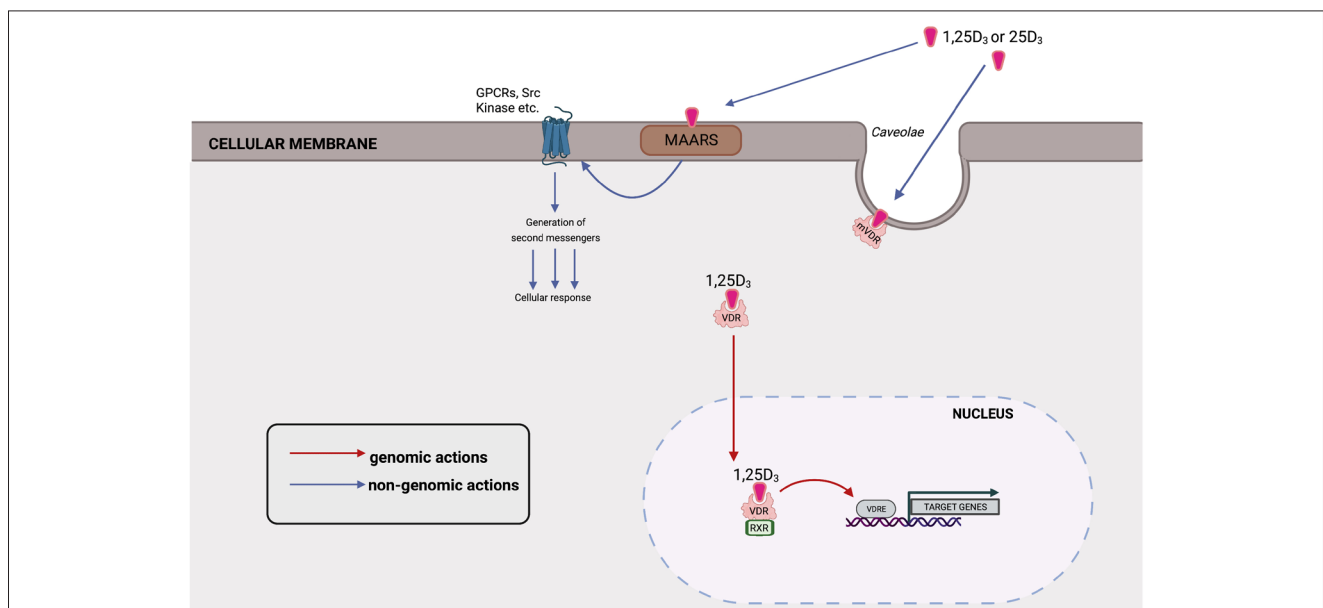
It regulates these functions via both VDR-mediated genomic pathways and membrane receptor-mediated rapid non-genomic responses. The ability to activate rapid membrane signalling has also been attributed to various steroid hormones, such as testosterone, oestrogens, aldosterone, and cortisol [29,42,52].

Even the major circulating metabolite of vitamin D, named 25(OH)D₃, has been shown to be capable of initiating rapid, non-transcriptional effects, such as a sustained and acute rise in intracellular Ca²⁺ levels, similar to what has been shown for the biologically active form of vitamin D₃ [42,53]. As proposed in this review, although knowledge of the area of rapid membrane-initiated non-genomic vitamin D-related activities has been constantly increasing, the relevance of these activities to physiological processes needs to be explored. In this regard, one of the major concerns is to recognise disorders that are exclusively due to a deficiency of the non-genomic effects of vitamin D.

The vitamin D endocrine system is primarily designed in processes that serve to maintain calcium and bone homeostasis, where rapid membrane-mediated non-genomic responses are probably not required. On the other hand, these rapid actions could play a significant role in other processes, including protection against DNA damage from ultraviolet light radiation and intestinal Ca²⁺ absorption. It has also been suggested that steroid molecules through their non-genomic effects and the consequent activation of signalling cascades might positively or negatively influence gene expression by acting on transcription factors [52], thus regulating the efficacy and potency of genomic actions [54]. For example, 1,25(OH)₂D₃ might lead to the generation of second messengers, such as PKC, PI3K, and PLA2, which in turn could modulate gene expression via gene regulatory elements or by using activated VDR [54,55].

In conclusion, recent years have seen significant progress in understanding of the endocrine system of vitamin D, expanding knowledge of vitamin D beyond its classic role in the regulation of calcium and phosphate homeostasis, and the prevention of rickets, osteomalacia and osteoporosis. Not only is it now well-established that the biological activity of vitamin D could be ascribed to its genomic activity, but calcitriol and more recently the 25(OH)D₃ have been described to trigger rapid non-genomic responses (Figure 1). Moreover, growing

Figure 1 The putative genomic and non-genomic actions of vitamin D. Red and blue arrows indicate respectively genomic and non-genomic pathways. Abbreviations: VDR: vitamin D receptor; RXR: retinoid X receptor; VDREs: vitamin D response elements; mVDR: membrane-bound VDR; MAARS: membrane-associated, rapid response steroid binding receptor; GPCRs: G protein-coupled receptors (image created by BioRender).



evidence has shown that vitamin D-related rapid non-genomic responses cannot be fully attributed to the activities of membrane-associated VDR, but are also linked to the existence of other membrane-associated vitamin D receptors, including Pdia3. Important issues related to the mechanisms underpinning membrane non-genomic effects remain to be elucidated. Furthermore, additional studies are needed to clarify the physiological and clinical significance of vitamin D-related rapid non-transcriptional effects, especially in persons suffering from hypovitaminosis D.

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