Abstract. To date, convergent data on the role of retinoic acid in the mature brain have established that this molecule, which acts as a hormone, helps to preserve cerebral plasticity by controlling dendritic spine density as well as hippocampal neurogenesis. A deterioration in cerebral plasticity seems to be at the base of the cognitive decline disease. Furthermore, the transcription of several genes, known as muted, in Alzheimer’s patients and whose transcripts are involved in the formation of senile plaques, are controlled by retinoic acid. As seen in other nutrients, aging leads to a lower production of retinoic acid; a phenomenon probably accentuated by the fact that Western populations consume an insufficient amount of vitamin A (60% of the population has a consumption lower than the recommendations [1]). These two phenomena (i.e. level of consumption, the lack of activation of vitamin A) accompanied by important individual differences, would help to explain why some patients have an almost normal aging process, whereas others gradually develop cognitive disorders and then, the disease. A better understanding of the role of a collapse of the retinoid status in the genesis of Alzheimer lesions could, beyond the definition of a preventive nutritional strategy, open therapeutic perspectives, through the use of molecules targeting the nuclear receptors.
Introduction

Retinoids, a family of compounds derived from vitamin A (Figure 1), have numerous important functions in many tissues, including: a role in vision, the maintenance of epithelial surfaces, immune competence, reproduction, and embryonic growth and development [2]. The majority of these functions (mechanisms underlying the extravisual functions) of vitamin A are performed by the vitamin A metabolite, retinoic acid (RA), which binds to receptors of the nuclear receptor superfamily, and regulates gene expression. It is well-known that retinoids, and particularly RA, play an important role during the normal development of the central nervous system (CNS) (see review by Maden [3]). Presently, the role of retinoids in the adult central nervous system is less conspicuous than their role in development, and has only recently attracted the attention of scientists. Some data suggest that the fine regulation of retinoid mediated gene expression seems fundamentally important for optimal brain functioning such as LTP, synaptic plasticity, learning and memory [4-9]. Recently, data from a number of studies have argued for the involvement of retinoid signaling in the etiology of Alzheimer's disease (AD) [10-12].

Figure 1. Chemical structures of retinoid family members.
**Vitamin A metabolism and transport (Figure 2)**

The capability for the *de novo* synthesis of compounds with vitamin A (retinol) activity is limited to plants and microorganisms. Therefore, higher animals must obtain vitamin A from their diet, either in the form of a preformed vitamin (the major dietary forms are long-chain fatty acid esters of retinol: retinyl ester or RE) or as provitamin carotenoids, such as β-carotene, α-carotene or β-cryptoxanthin, found in plant-derived foods. Presently, it is recommended that 60% of the vitamin A intake is in the form of carotenoids (plant sources) and 40% in the form of retinols (animal sources). RE must be hydrolysed prior to intestinal absorption. The absorption efficiency is higher for preformed vitamin A (80-90%) than for carotenoids (50-60%). Carotene is converted to retinol in the intestinal mucosa via two enzymatic steps. Free retinol is reesterified in the mucosal cells by the enzyme lecithin:retinol acyltransferase (LRAT); the resulting RE are incorporated into chylomicrons and absorbed via the lymphatics (see review by Harrisson [13]). Under normal nutritional conditions, most of the vitamin A in an organism is stored in the liver (essentially as RE forms), partly in the hepatocytes and in higher amounts, as lipid droplets in the stellate cells (also called Ito cells), from where vitamin A will be mobilized when needed [14]. RE are hydrolyzed prior to mobilization from the liver and free retinol is complexed to retinol binding protein 4 (RBP4). Approximately 95% of the retinol-binding proteins (RBP) circulate in plasma as a macromolecular complex with another transport protein, thyroxine hormone carrier transthyretin (TTR). Retinol is taken up by target cells through an interaction with the membrane receptor for RBP4 (STRA6).

It then enters into the cytoplasm, where it binds to cellular retinol binding protein 1 (CRBP1) and, in a two-step process, is metabolized to all-trans retinoic acid (atRA), the active metabolite of vitamin A [2]. The rate limiting step in this process is the oxidation of retinol to retinal, and the final step, is the oxidation of retinal to retinoic acid (RA). Cytosolic Medium-Chain Alcohol Dehydrogenase (ADH), and more precisely ADH1, ADH3 and ADH4, are involved in the oxidation of all-trans retinol to all-trans retinal. The oxidation of retinol to retinal also appears to be catalyzed by members of the Membrane-Bound Short-Chain Dehydrogenase/Reductase (SDR) family of microsomal enzymes, including RDH1, RDH5, RDH11, CRAD1, CRAD2, CRAD3 and retSDR1 [15]. The oxidation of all-trans retinal to all-trans retinoic acid is catalyzed by Retinal Dehydrogenase (RALDH), and more precisely by the isoforms 1, 2, 3 and 4. The catabolism of RA is an important mechanism for controlling RA levels in cells and tissues.
Figure 2. The retinoid cascade.

Cellular retinoic acid binding proteins I and II (CRAPBI and II) are the cytoplasmic-binding proteins for RA. One function of these proteins may be to transport RA into the nucleus in order to mediate its effect by either inducing or repressing gene transcription by binding to specific nuclear receptors which function as transcription factors: RAR (whose ligands are the all-trans RA and 9-cis RA isomers) and RXR (whose ligand is the 9-cis RA
The fact that RA activates nuclear transcription factors was discovered in 1987 [17, 18]. The RA receptors belong to the same family as proliferators-activated receptors (PPAR), vitamin D receptors (VDR), thyroid hormone receptors (TR) and steroid receptors. In the presence of a ligand, the receptor switches in conformation and releases corepressors that would otherwise keep the gene repressed in the absence of a ligand. This is followed by a modification in the decompaction of chromatin structure, allowing the transcriptional machinery to gain access to the promoter and to initiate the transcription. The RA response element (RARE) binds the receptor dimers [19]. Most often, RAR is dimerized to RXR, which has the particularity to act independently of the presence of its ligand [20]. RAR and RXR each have three genes (alpha, beta and gamma) and gene splicing significantly increases the number of variants [21]. RAR and RXR also have three isotypes each (α, β and γ), which are encoded by distinct genes. Thus, for each RAR isotype, there are several isoforms generated by differential promoter usage and splicing [22, 23]. The multiple RAR and RXR isotypes and isoforms are conserved in vertebrate evolution and display distinct spatiotemporal expression patterns in developing embryos and adult tissues, suggesting that each receptor performs a unique function [24].

Therefore, vitamin A and more precisely, RA via its specific nuclear receptors, play a critical role in a variety of essential life processes, including reproduction, embryonic development and modulation of the growth and differentiation of a wide variety of mammalian cell types. It has been suggested that RAR/RXR signaling regulates approximately 500 genes [2]. A much lower number was experimentally shown to be activated via the classical RARE driven pathway, whereas many cases of gene suppression have been described. Some authors have shown non-genomic modes of RA action. These rapid actions include the regulation of gap junctions [25], spinule formation in the retina [26], an effects on dendritic spines in the hippocampus [27]. Another pathway of action is the repression of AP-1 (Jun, Fos) activity, which involves RAR/RXR dimmers, but not RARE [28].

**Vitamin A and the adult brain**

1/ Retinoic acid signaling in the brain

During brain development, vitamin A, and more precisely RA, plays a key role by regulating patterning, neurogenesis, neural specification, and neurite outgrowth. Recent studies have suggested that retinoids may also play an important role in the adult central nervous system [29-31]. In the adult brain, regions that exhibit RA signaling are regions of high neuronal
plasticity i.e. the hippocampus, medial prefrontal cortex and retrosplenial areas [32]. Several lines of evidence point to the importance of RA in the functioning of the striatum and nucleus accumbens. It has been shown that (i) cellular retinoic acid and retinol binding proteins and high levels of nuclear retinoic acid receptors have been observed in these areas [33, 34] and (ii) the striatum presents the highest levels of RA [35]. Several authors have shown that the striatum synthesizes RA and contains much of the biochemical apparatus associated with RA responsiveness and metabolism [36-38].

Among the RA nuclear receptors, RARβ is the main isoform expressed in the mature brain [39]. Moreover, RXRβ and RXRγ are also expressed at high levels in the brain [40]. Thereby, the co-expression of RARβ and RXRβ/γ suggests that these receptors may contribute to specific functions in the central nervous system by modulating the expression of their target genes. Among the many genes whose expression is regulated by RA, there are those coding for their own nuclear receptors and those coding for neuron-specific proteins involved in many activities in the mature brain, e.g., synaptophysin, nerve growth factor, N-methyl-D-aspartate receptor, dopamine receptor 2, choline acetyltransferase, neurogranin, neuromodulin. Finally, retinoic acid and its receptors also regulate quite a lot of genes coding for proteins implicated in neurodegenerative processes, such as the APP protein and the tau protein.

It is currently accepted that retinoic acid plays a dominating role in the preservation of cerebral functionality. Thus, it is of first importance to study the effects of the status in vitamin A, or in retinoic acid, in the adult brain, and more particularly during aging. Indeed, recent data have suggested that changes in retinoids are capable of producing alterations in neuronal target proteins and consequently may affect physiological maintenance processes in the mature brain [41]. Alterations of cerebral plasticity and memory deficits have been described in vitamin A depleted animals as described in a latest part. Moreover, it has been shown that RARβ and RARβ-RXRγ knockout mice display an alteration of LTP, as well as substantial performance deficits in a hippocampal-dependent spatial learning task [5, 42]. RARβ mutations, with either RXRβ or RXRγ, result in impaired locomotion typical of abnormal striatal function and possibly related to a decrease in the dopamine D1 and D2 type receptors in striatal neurons [43, 44]. Chronic ethanol consumption, which produces cognitive deficits, also induces disorders in RA biosynthesis; ethanol can induce RARβ and RXRβ/γ expression in vivo, and blocking RARβ activity was shown to reverse an alcohol-induced working memory deficit. Thus, the over-expression of brain RA nuclear receptors seemed to be involved in memory impairments observed during chronic
ethanol consumption (Alfos et al., 2001). Astrogial-derivated RA may even be an important signal for neurogenesis in the dentate gyrus of the hippocampus [45].

Together, these results show that either nutritional or physiological situations involving modifications of the level of expression of the brain retinoid nuclear receptors lead to considerable neurobiological alterations and to mnemonic deterioration.

2/ Vitamin A and aging

Age-related alterations in vitamin A metabolism, particularly plasma retinol concentrations, have been reported in both rats and humans [46, 47]. More recently, studies have shown that a moderate down-regulation of retinoid mediated transcription events occurs naturally with senescence. A lower abundance of RARβ and RXRβ/γ mRNA has been effectively observed in the whole brain and hippocampus of aged mice and rats. Generally, authors have attributed this decrease in nuclear receptor expression to a reduction in the bioavailability of RA associated with aging. This explanation seems to be confirmed by the fact that a significant decrease in retinol concentration was observed in the serum of aged rats. The administration of RA restores the age-related decrease in mRNA levels to their presenescence levels [4, 48, 49]. Finally, more recently, retinoid hyposignaling, as evidenced by a hypoexpression of retinoid receptors, has been reported in human peripheral blood mononuclear cells [50].

The hypoactivity of retinoid signaling observed in aged animals was associated with a decreased expression of genes encoding for neural proteins and implicated in synaptic plasticity: neuromodulin (GAP-43) and neurogranin (RC3) [51]. The age-related decreased expression of RC3 observed in mice was correlated with a LTP deficit and severe age-specific memory impairment. RA administration to aged animals restores the mRNA level of target genes involved in synaptic plasticity, and concomitantly alleviates both the hippocampal LTP and relational memory seen in aged mice [6]. Vitamin A supplementation was shown to counteract the aging-related hippocampal hypoexpression of GAP-43, as well as the short term/working memory deterioration and alleviated the long-term declarative memory impairment [42]. Ethanol consumption in aged mice also reversed the age-related hypo-expression in brain RARβ and the target genes RC3 and GAP-43 [52] and reduces a selective age-associated memory deficit in mice [53].

Together, these data suggest that a fine regulation of retinoid mediated gene expression is fundamentally important for optimal brain functioning during aging and for the maintenance of memory performances. This potential
role of retinoids has been argued in works studying the functional consequences of a decrease in the bioavailability of vitamin A generated by a vitamin A deficiency (VAD).

3/ The vitamin A deficiency model

VAD that is characterized by a reduced expression of brain-specific retinoid nuclear receptors in control animals results in a decline in the activity of two target genes involved in synaptic plasticity (Neurogranin/RC3 and Neuromodulin/GAP43) as well as in a selective behavioral impairment similar to that observed in aged mice [9, 39, 54-57]. In rats subjected to 14 weeks of VAD, there is a decrease in cell proliferation and neurogenesis concomitant to spatial learning and memory deficits. More importantly, these effects are reversed after four weeks of RA treatment [58]. Moreover, adult rats maintained on a vitamin A-free diet for 12 months developed a severe deficit in spatial learning and memory, and this cognitive impairment was fully restored when vitamin A was once again available in the diet [7, 8].

These few studies have revealed neurological alterations associated with VAD. Experiments using mouse models have provided evidence that, contrary to aged mice, the administration of RA to vitamin A-deprived animals failed to fully normalize the expression of RC3 and had no effect on relational memory [9]. Knowing that (i) RC3 is not only under the influence of retinoids [59], but is also regulated by thyroid hormones (whose active metabolite is triiodothyronine, T3) [60, 61] and (ii) in consideration of the close relationship between the activity and signaling pathways of retinoids and thyroid hormones previously described in VAD [62, 64], it has been suggested that thyroid disorders are involved in the inefficacy of RA to restore neurological alterations. Recent results have strengthened this hypothesis in that the alteration of the T3 signaling pathway associated with VAD has been shown to be a limiting factor that impedes RA from exerting its modulating effect [57].

The vitamin A deficiency model both highlights and describes the involvement of vitamin A signaling in cerebral plasticity and memory performance.

Beyond its implication in the implementation of the cognitive deficits during aging, hypoactivity of the retinoid signaling pathway could also influence the late onset of Alzheimer’s disease (AD).

Vitamin A and Alzheimer’s disease

AD is the most common cause of dementia in the elderly. This chronic neurodegenerative disease is characterized by the progressive deterioration of
cognitive functions including memory, judgment, language skills, decision-making, orientation, etc. Clinical symptoms include alterations in neural plasticity (e.g. the loss of selective neurons and synapses), extracellular senile plaques containing amyloid-β peptide (Aβ) deposits, as well as intraneuronal neurofibrillary tangles. The involvement of RA in cognitive functions and neuronal plasticity has previously been described. Recently, data from a number of studies have argued for the involvement of retinoid signaling in the etiology of AD. On one hand, Goodman investigated the genetic links between RA signaling and AD since gene loci thought to be involved in AD were clustered around genes for CYP26 enzymes (cytochrome P450 enzymes involved in the catabolism of RA for controlling RA levels in tissues), RARα, RXRβ, RXRγ, CRABP-II, and RBP. On the other hand, a decrease in serum retinol levels has been revealed in patients with AD, and it has been hypothesized that a decrease in the availability of RA and a subsequent dysregulation of retinoid genes and their target genes contribute to late-onset AD [10, 65-67].

1/ Vitamin A transporters and AD

Apolipoprotein E (ApoE), a transport protein for RE in chylomicron, is the major apolipoprotein in cerebrospinal fluid and has been identified as a major susceptibility gene in AD [68]. The ApoE ε2 allele, which is associated with a decreased risk of AD in humans and memory impairment in rats, has a better ability to carry retinoids [10]. In neuronal cells, the abundance of constitutively expressed ApoE is lower following RA treatment [69], while in rat primary astrocyte cultures, RA increases APOE secretion [70]. Levels of the lipocaline apolipoprotein D, another transporter protein of retinol in the CNS, are increased in the neurons of AD patients. RA regulates its expression.

2/ Vitamin A and neurofibrillary tangles

Among the genes potentially regulated by RA, there is one gene that codes for microtubules-associated-protein-tau (MAPT) and which is preponderant in the formation of the neurofibrillary tangles [71-73].

3/ Vitamin A and β-amyloid (Aβ) (Figure 3)

Aβ is a mixture of heterogeneous peptides derived from the amyloidogenic pathway, including two sequential endoproteolytic cleavages of the β-amyloid precursor protein (APP) catalyzed by two distinct enzymes
Figure 3. Retinoid acid and the amyloidogenic pathway.

referred to as β- and γ-secretase [74], [75]. The β-site cleaving enzyme (BACE1) is highly expressed in brain tissue and colocalizes with intracellular Aβ production sites. The cleavage of APP by this enzyme results in the release of sAPPβ into the extracellular space. The subsequent cleavage of the fragment remaining in the membrane by γ-secretase, releases an intact Aβ peptide, a component of extracellular amyloid plaques. APP can also be processed by a non-amyloid pathway in physiological conditions. This secretory pathway includes the cleavage of APP by a putative α-secretase in the Aβ region, thereby precluding the formation of Aβ. This type of α-secretase activity has been attributed to the metalloproteinases ADAM9 and ADAM10 [76].

It had been shown that the disruption of retinoid signaling causes the deposition of β-amyloid in the adult brain [11]. These authors have documented an amyloid β deposition in the brains of the 1-year-old retinoid deficient rats. Similarly, vitamin A (or retinoid) inhibits and destabilizes preformed Aβ aggregates and consequently protects against plaque formation, probably via its nuclear receptors [77, 78]. To date, there are several biochemical data that support the involvement of vitamin A signaling in the formation of Aβ. Indeed, key steps of the amyloid production process...
are under the control of proteins whose expression is positively regulated by RA in vitro, including: APP, the β-site APP cleaving enzyme (BACE or β-secretase), presenilin 1 and 2 (PS1 and PS2), two of the complex γ-secretase proteins (their up-regulation by ATRA might promote plaque formation), as well as ADAM10 [29, 79-81]. Interestingly, an in vitro study revealed that RA treatment increases the ADAM10 protein level much more than that of BACE, suggesting a shift in APP processing toward the α-secretase pathway in response to RA [82, 83]. The insulin degrading enzyme (IDE), a metalloprotease enzyme responsible for insulin degradation, has been shown to play a key role in Aβ peptide degradation both in vitro and in vivo, and is selective for the Aβ monomer. IDE has been observed in human cerebrospinal fluid (CSF). Its activity, levels, and mRNA are decreased in AD brain tissue and are associated with increased Aβ levels [84-87], suggesting that the modulation of IDE activity may alter the risk for AD. IDE contains a RARE response element in its promoter and the transcription of IDE is positively regulated by RA [88].

In order to consider the use of retinoid therapy for AD, it is necessary to establish the precise role of vitamin A and its receptors in vivo in the multiple processes involved in regulating plaque formation.

As stated above, recent data have provided evidence that VAD was responsible for instigating an alteration of the neuronal plasticity as well as inducing cognitive impairment [9, 56, 58]. Additional data have revealed a vitamin A-deficiency-related dysregulation of the amyloidogenic pathway in the cortex of rats, which is known to be the first brain area altered by AD development: the first stage of Aβ deposition begins exclusively in the neocortex before expanding to other regions [89, 90]. Authors have shown that hypo-activity of the retinoid signaling pathway leads to (i) an increase in the APP770-751/APP695 ratio, which is an important clue to the etiology of amyloid deposition and also of plaque formation in AD patients [91], and (ii) a decreased expression of APP695, BACE and APP-CTF in whole brain and cortex of rats fed a vitamin A-free diet for 13 weeks. There is a longstanding controversy as to which APP transcript is up- or down-regulated in amyloidogenic diseases. Nevertheless, the loss of APP695, a brain-specific isoform, has been described in the cortex and hippocampus of AD patients [91, 92]. The phosphorylated forms of APP-βCTF and APP-β’CTF seem to play a determining role in CTF processing leading to neuritic plaque formation, by facilitating their own cleavage into Aβ by γ-secretase [93]. In this way, the reduced levels of phosphorylated β- and β’CTF, which occurred in the cortex when there was a vitamin A deficiency for an extended period of time, might be due to a rise in γ-secretase activity and thus, might reveal an increase in Aβ formation. Indeed, some studies have shown an inverse relationship between γ-secretase and α- and βCTF: (i) an elevation in
the steady-state levels of \( \alpha \) - and \( \beta \)CTF in PS1 gene knockout mice (a member of the \( \gamma \)-secretase complex) [94], and (ii) an accumulation of the \( \gamma \)-secretase product (\( \gamma \)CTF) and a concomitant reduction in the levels of both \( \alpha \) - and \( \beta \)CTF in different cell lines that over-express PS1 [95]. Moreover, the decreased amount of fragment \( \alpha \)CTF (an \( \alpha \)-secretase product generated during the physiological pathway) noted in the cortex of vitamin A-deficient rats might reflect an underlying neuropathic process, whereby APP695 is converted to \( \beta \)CTF and thus, A\( \beta \), rather than to \( \alpha \)CTF. In fact, such a process has already been described in AD patients [96]. This hypothesis was supported by recent reports suggesting a RA-associated shift in APP processing toward the alpha secretase pathway [81, 82].

Finally, very recently, several authors have reported results supporting all-\( \text{-trans} \)-retinoic acid (ATRA) as an effective therapeutic agent for the prevention and treatment of AD. In amyloid precursor protein (APP) and presenilin 1 (PS1) double-transgenic mice, the administration of ATRA induced a robust decrease in brain A\( \beta \) deposition and tau phosphorylation. This effect was accompanied by a significant down-regulation of tau phosphorylation [97].

These results complement earlier data indicating the involvement of retinoid signaling in the etiology of Alzheimer diseases and argue for the potent anti-amyloidogenic effect of vitamin A suggested, to date, by \textit{in vitro} studies. Nevertheless, the underlying molecular mechanisms are incompletely understood.

**Conclusion**

Together, these data suggest that a fine regulation of retinoid mediated gene expression seems fundamentally important for optimal brain functioning and argue that vitamin A has an important role, via its nuclear receptors, in the multiple processes involved in regulating plaque formation. In a perspective of a nutritional prevention of Alzheimer disease, it will be of first importance to better understand the involvement of the age-related hypoactivity of the vitamin A signaling pathway in the genesis of pathologic lesions.

Besides retinoic receptors (RAR), the steroid/thyroid nuclear receptor superfamily includes other transcriptional factors relevant for neurodegenerative diseases. This is the case for peroxysome proliferator-activated receptors, which also form heterodimers with RXR in order to bind to their response elements (PPRE) and active gene transcription [98]. Currently, several studies have described a much closer relationship between RA and fatty acid signaling pathways, since the ability of several polyunsaturated fatty acids (PUFA) to specifically bind and activate RXR at supra-physiological levels has recently been shown [99, 100]. It is now well accepted that these nuclear
receptors are master transcription factors which, by the means of a precise combination, orchestrate the maintenance of neurobiological properties which underpin memory processes. Moreover, it is known that these receptors are also sensors of vitamin or lipid content in the diet and control its metabolic response. It seems to be of first importance to consider vitamin A and fatty acid signaling pathways together, since it has now been established that the dietary intake of PUFA and, more importantly, the total intake of calories from fat leads to an accumulation of neurodegenerative markers [101]. Thus, it is possible to assume that modifications in the brain bioavailability of these modulators (RA or fatty acids) -via a lesser dietary content or by an age-related physiological decline in the capability to activate these modulators- may rapidly induce changes in the pattern of nuclear receptor expression, and consequently, lead to neurobiological deterioration and neurodegenerative processes.

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