Functional Implication of the Vitamin A Signaling Pathway in the Brain

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Vitamin A is necessary for normal embryonic development, but its role in the adult brain is poorly understood. Vitamin A derivatives, retinoids, are involved in a complex signaling pathway that regulates gene expression and, in the central nervous system, controls neuronal differentiation and neural tube patterning. Although a major functional implication of retinoic signaling has been repeatedly suggested in synaptic plasticity, learning and memory, sleep, schizophrenia, depression, Parkinson disease, and Alzheimer disease, the targets and the underlying mechanisms in the adult brain remain elusive.

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Since the original description of the congenital ocular malformation due to vitamin A deficiency,1 many clinical and experimental findings have indicated that vitamin A and its active derivatives, retinoids, play a major role in embryogenesis, cell proliferation and differentiation, and apoptosis.2,3 Aside from vitamin A deficiency,4 high doses of retinoids also induce developmental abnormalities.5 However, the physiologic role of retinoids cannot be extrapolated from these extreme conditions.6 Retinoic acid (RA), the most active derivative of vitamin A, can rescue most defects caused by vitamin A deficiency. It acts through its binding to nuclear retinoid receptors, which are ligand-activated transcription factors.7 Discrete brain regions express several proteins that transduce the RA signaling pathway, including metabolic enzymes, binding proteins, and nuclear receptors.8 Whether this complex pathway affects adult brain functions through controlling events during development or through continuous local regulatory actions on gene expression, neurotransmission, and neurotrophic factors may be difficult to elucidate, especially because the 2 possibilities are not exclusive. Recent findings in sleep, synaptic plasticity, and aging, reviewed herein, provide compelling evidence that the transduction of the RA signal by nuclear retinoid receptors plays a major role in adult brain functioning.

VITAMIN A AND RETINOID SIGNAL TRANSDUCTION

Vitamin A is provided either in lipid-soluble form by animal-derived foods or in water-soluble form by plant-derived foods (Figure 1). Excess lipid-soluble vitamin A (preformed vitamin A) accumulates in body fat stores and in the liver and is associated with toxic effects, whereas excess water-soluble provitamin A (eg, beta carotene) can be excreted and is usually not associated with toxic effects. The liver is the major site of storage and processing of vitamin A in the forms of retinyl esters and retinol, respectively. To meet tissue requirements, retinol is secreted from the liver, bound to the retinol binding protein, and transported through blood circulation toward the target organ. Very recently a membrane receptor was discovered with high affinity for retinol that is responsible for retinol uptake by cells.9 The tissue distribution of all-trans RA results from the balancing activities of cellular enzymes, including RA-

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The central nervous system (CNS) developmental defects caused either by vitamin A deficiency or excess or by defects in RAR/RXR genes, their metabolic enzymes, and binding proteins have been extensively reviewed.\textsuperscript{6,12} In addition to the well-established role of RA in anteroposterior and dorsoventral patterning of the neural tube, recent findings\textsuperscript{13,14} established that RA has highly specific effects on the neuronal differentiation of precursor and embryonic stem cells. There is an apparent continuity of the RA effects between neural precursor cells with glial characteristics in specific brain regions (eg, the forebrain) throughout life.\textsuperscript{13}

The role and expression of genes involved in RA signaling are developmentally restricted. Although RXRs show specific expression patterns in the adult brain, they likely act mainly as heterodimeric partners for RARs and other nuclear receptors because it is questionable whether their ligand 9-cis RA is actually produced in vivo.\textsuperscript{6} The transcripts for RAR\textsubscript{x}, RAR\textsubscript{B}, and RAR\textsubscript{y} are widely expressed in the brain, but the corresponding proteins do not show correlated localization patterns, suggesting posttranscriptional regulation events.\textsuperscript{8} Except for RAR\textsubscript{y}, which displays uniform low levels of protein expression in most brain regions, other RARs exhibit specific patterns of expression. The RAR\textsubscript{x} is mainly expressed in the hippocampus, thalamus, and pons, whereas RAR\textsubscript{B} is the most abundant RAR in the striatum, hypothalamus, and medulla. The expression pattern of RAR in the adult brain is quantitatively and qualitatively different from that in the developing and neonatal brain,\textsuperscript{16} suggesting specific functional, although poorly understood, roles in the adult brain.\textsuperscript{17}

**RETINOID SIGNAL TRANSDUCTION, LEARNING, MEMORY, SYNAPTIC PLASTICITY, AND AGING**

Loss of function of RAR\textsubscript{B}, RXR\textsubscript{y}, or both (compound Rarb/Rxrg-null mutants) in the mouse induces defi-
ciency in special memory formation, as tested using the Morris water maze.18,19 Similar defects in spatial learning and memory have been reported in vitamin A–deficient rats, all of which are restored after the addition of vitamin A to the diet.20 Vitamin A deficiency in adult mice is also associated with decreased expression of RARs, RXRs, and neurogranin messenger RNA that could be reversed, parallel to improvement in memory deficit, by RA treatment.21 The striatum seems to be a brain region highly sensitive to vitamin A bioavailability, as changes in gene expression on vitamin A deficiency are most reliably detected in this brain region.22 Whether excess RA affects learning and memory is less studied. Adult rats and mice treated with 13-cis RA perform poorer in an 8-arm radial maze,23 but this finding is questionable because it could not be replicated recently.24 Spatial and recognition working memory was also studied in detail in Rarb- and Rxrg-null mutant mice. Compound null mutants display deficits in spatial memory, although they can learn spatial place preference or spatial orientation. They are also deficient in novel object recognition at intermediate delay.25 Because Rxrg-null mutants exhibit deficits similar to compound Rarb/Rxrg-null mutants, RXRγ is likely to be the functionally predominant receptor involved in working memory.25 At the cellular level, hippocampal long-term potentiation and depression represent the underlying biological substrates of learning and memory, and it has been shown that vitamin A deficiency induces long-term potentiation deficits that can be reversed by RA treatment.19 Accordingly, Rarb- and Rxrg-null mutant mice display impaired long-term potentiation.18

Age-related changes in vitamin A signaling, especially reduced plasma retinol concentrations and decreases in RAR and RAR messenger RNA, have been reported in human and animal models.26,27 Huguier and Jaffard's group in France conducted a series of experiments27-32 to investigate the potential role of the retinoid pathway in aging and the associated cognitive deficits. They mainly focused on the expression patterns of RARs and RXRs and their target genes, such as neurogranin, in rats and mice. They showed that the expression of all these genes decreases with aging, a phenomenon that could be reversed by RA treatment.27 These age-related decreases in expression are associated with altered cognitive functions (eg, learning and memory).28,29 Conversely, chronic alcohol administration in mice induces overexpression of the same genes, an effect that can be reversed by treatment with an RAR antagonist.30 Also, moderate alcohol administration restores expression of RARs and their RA target genes in aged mice, a situation that may have beneficial effects on cognitive alteration during senescence.31,32

RETINOID SIGNAL TRANSDUCTION AND BRAIN RHYTHMIC ACTIVITY

The electroencephalogram (EEG) is one of the most efficient tools for indexing brain functional variations, and it shows dramatic changes with vigilance states. The EEG of non-rapid eye movement sleep (NREMS) is characterized by high-amplitude slow oscillations called slow wave or delta activity (0.5-4.5 Hz). Delta activity is directly associated with sleep need, intensity, and continuity, and it presents predictable changes with previous wakefulness duration. Delta activity is, thus, believed to reflect a homeostatic process underlying the recovery function of sleep. Slow oscillations are generated by thalamocortical feedback loops or in the cortex.33 Franken et al34 previously showed in mice that several sleep EEG characteristics are strongly affected by genetic factors. The frequency of the theta waves (5-8 Hz), which characterize paradoxical sleep, and the relative contribution of delta and theta activities to the NREMS EEG are among the most heritable phenotypes in mice,34,35 and they may be controlled by single genes.36 As opposed to most inbred mouse strains, in DBA/2J (D2) mice the EEG activity during NREMS shows a clear predominance in the theta, but not the delta, frequency range. Sleep is abnormally fragmented in these mice, and the rate at which sleep need accumulates is significantly reduced compared with most other inbred strains.34,37

We used the theta to delta ratio during NREMS EEG to map a major locus linked to this peculiar sleep phenotype on mouse chromosome 14 (Figure 2A). The best candidate gene responsible for the phenotype is Rarb, for which segregating polymorphisms are observed in the 5’ untranslated region of the transcript coding for the Rarb isoform. In addition, Rarb messenger RNA is expressed at a higher level in the brain of D2 mice and in a few other strains displaying a similar NREMS EEG phenotype (Figure 2B), whereas Rarb-null mice have increased delta activity.38 As mentioned previously herein, changes in RAR expression with subsequent changes in cognitive functions have been documented in many conditions, including aging, RA treatment, and alcohol administration. Thus, we tested whether sleep deprivation, which has a dramatic effect on NREMS EEG findings, similarly affects the expression of Rarb. Six hours of sleep deprivation actually doubles Rarb1 and Rarb2 expression in the brain (Figure 2B). Recovery sleep after sleep deprivation is characterized by a significant increase in delta and theta activity without major changes in their relative contributions. Linkage analysis for the NREMS EEG phenotype during recovery sleep points to the same region of chromosome 14 containing Rarb (Figure 2A), strengthening the robustness of the association between the Rarb gene and reduced delta activity. As mentioned previously herein, RARB is mainly expressed in the striatum, hypothalamus, and medulla and is paradoxically undetectable in the thalamus and cortex,8 the 2 main structures involved in EEG delta activity.33 Along these lines, Rarb-null mice display impaired hippocampal long-term potentiation,17 but RARB is not detected in the hippocampus,4 suggesting either that low levels of RARB (below the detection limit using standard methods) might have significant effects or that the sites of RAR expression are poorly correlated with the sites of RA action. Another possibility is the involvement of
RARβ-dependent developmental effects on CNS plasticity or on patterning of dopaminergic neurotransmission. Actually, NREMS emerges during the early postnatal period and reaches its adult characteristics after adolescence, in parallel to changes in RAR gene expression. Finally, although the role of the striatum in NREMS is not clear, a direct effect of RARβ on dopaminergic neurotransmission may contribute to the NREMS EEG changes observed in D2 mice. The developmental changes in sleep and RAR gene expression, and the effects of vitamin A deficiency or excess on sleep, are currently under investigation.

**RELEVANCE TO CLINICAL PRACTICE**

Besides the teratogenic effects of vitamin A excess or deficiency during development, evidence accumulates to indicate involvement of the RA signaling pathway in several major CNS disorders. As discussed previously herein, RA-dependent pathological conditions might be complex, owing to nonexclusive developmental or adult-specific effects. Because RA is involved in amyloid plaque formation and evidence has been found linking late-onset Alzheimer disease and chromosomes 10q23 and 12q13, which contain genes involved in RA signal transduction, including, notably, Rarg, retinoid signaling was implicated in Alzheimer disorder. On the one hand, in vitro studies indicate that RA-induced differentiation of neuroblastoma cells is associated with up-regulation of presenilins 1 and 2 and with a more than 10-fold induction of amyloid protein precursor messenger RNA. On the other hand, vitamin A has an antioxidant property that may explain its anti-amyloidogenic effect in vitro. This finding is corroborated by the observation that rats with vitamin A deficiency show accumulation of amyloid β protein in their cortex and that such amyloid β protein deposits can be cured by RA administration. Whether the RA signaling pathway actually plays a major role in the pathophysiologic mechanisms of Alzheimer disease has not yet received a clear answer. Neverth-
theless, the blood level of vitamin A is reduced in patients with Alzheimer disease, whereas expression of RARα and of the RA-synthesizing enzyme retinaldehyde dehydrogenase 2 is decreased in the cortex and meningeal vessels of postmortem Alzheimer disease brains.

Several lines of evidence suggest a link between the RA signaling pathway during development and schizophrenia, which is considered to be related to as yet unidentified neurodevelopmental events resulting in ventricular enlargement and structural alteration in the frontal cortex, amygdala, hippocampus, temporal lobes, and cingulated gyrus. In addition, the dopaminergic system, which constitutes a well-documented pathway involved in schizophrenia and Parkinson disease, is one of the best-established targets of RA action in the CNS during development and adulthood. The promoter of the D2 dopamine receptor gene contains a functional RAR/RXR binding motif, and the expression pattern of dopamine D1 and D2 receptors is reduced in compound Rarb/Rxrb-null and Rarb/Rxrg-null mutant mice. The implication of retinoid signal transduction in controlling dopaminergic neurotransmission is further strengthened by the presence of high levels of RA-synthesizing enzymes in the mesencephalic dopamine system and by the fact that RXR and its heterodimeric partner Nur77 are believed to critically control the survival, adaptation, and homeostatic regulation of the dopaminergic system.

In mice, RA controls the differentiation of serotonergic neurons and the expression of serotonin 1A receptors, indicating a possible link between RA signal transduction and the serotonergic system, with implications for sleep and mood regulation. Depression is characterized by major abnormalities in sleep, including reduced delta activity, for which the underlying biological mechanism remains largely unknown. Excess vitamin A has been associated with depressive mood and sleep disorders that are similar to those observed on treatment with the synthetic retinoid isotretinoin to cure nodular acne. However, a direct link has never been firmly established. Finally, when the NREMS EEG in mice is dominated by theta instead of delta activity, the mouse strains are highly susceptible to audiogenic and absencelike seizures. Similar to excess RA, antiepileptic drugs can induce developmental defects, which are possibly mediated through an increase in the plasma vitamin A concentration. Findings in experimental models, such as rats and mice, suggest a major role for the dopaminergic system in the control of seizure activity, consistent with the hypothesis that changes in sleep EEG findings (decreased delta and increased theta activity) might partly be associated with the regulatory effects of the RA signaling pathway on the mesolimbic dopaminergic pathway.

CONCLUSIONS

All of the conditions reviewed herein are complex and might be controlled by genetic and environmental factors. Similar to most complex traits, they involve critical periods during development, and most of the associated disorders emerge from subtle developmental defects. The functional implication of the retinoid signaling pathway is strongly substantiated by its multiple and complex roles during CNS development and in the adult brain. The recent finding that the RA signaling pathway is involved in sleep regulation further raises 2 interesting possibilities. First, the NREMS EEG phenotype we discovered is functionally relevant to synaptic plasticity, aging, and all pathological conditions reviewed herein because delta activity can be used as a biological marker for schizophrenia, depression, Parkinson disease, and Alzheimer disease. Whether changes in the retinoid signaling pathway can be indexed through recording the NREMS EEG under various experimental and pathological conditions in humans needs further investigation. Second, the major sleep-related change in the RA signaling pathway applies to the expression of Rarb, for which the biological consequences are unknown. However, this may have potential clinical implications because Rarb expression in the brain can be rapidly modified by behavioral states (eg, a few hours of sleep deprivation).

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REFERENCES

27. Enderlin V, Pallet V, Alfos S, et al. Age-related de-

24. Ferguson SA, Berry KJ. Oral Accutane(R) (13-


15. Haskell GT, LaMantia AS. Retinoic acid signaling

13. McCaffery P, Drager UC. Regulation of retinoic acid

10. Blomhoff R, Blomhoff HK. Overview of retinoid me-

11. Laudet V, Gronemeyer H.

15. Ko¨nig G, Masters CL, Beyreuther K. Retinoid acid

17. Lane MA, Bailey SJ. Role of retinoid signalling in the

12. Maden M. Retinoid signalling in the develop-

10. Blomhoff R, Blomhoff HK. Overview of retinoid me-

11. Laudet V, Gronemeyer H. The Nuclear Receptor


15. Haskell GT, LaMantia AS. Retinoic acid signaling

13. McCaffery P, Drager UC. Regulation of retinoic acid

10. Blomhoff R, Blomhoff HK. Overview of retinoid me-

11. Laudet V, Gronemeyer H.

15. Ko¨nig G, Masters CL, Beyreuther K. Retinoid acid

17. Lane MA, Bailey SJ. Role of retinoid signalling in the

12. Maden M. Retinoid signalling in the develop-

10. Blomhoff R, Blomhoff HK. Overview of retinoid me-

11. Laudet V, Gronemeyer H.

15. Ko¨nig G, Masters CL, Beyreuther K. Retinoid acid

17. Lane MA, Bailey SJ. Role of retinoid signalling in the

12. Maden M. Retinoid signalling in the develop-

10. Blomhoff R, Blomhoff HK. Overview of retinoid me-

11. Laudet V, Gronemeyer H.

15. Ko¨nig G, Masters CL, Beyreuther K. Retinoid acid

17. Lane MA, Bailey SJ. Role of retinoid signalling in the

12. Maden M. Retinoid signalling in the develop-

10. Blomhoff R, Blomhoff HK. Overview of retinoid me-

11. Laudet V, Gronemeyer H.

15. Ko¨nig G, Masters CL, Beyreuther K. Retinoid acid

17. Lane MA, Bailey SJ. Role of retinoid signalling in the

12. Maden M. Retinoid signalling in the develop-

10. Blomhoff R, Blomhoff HK. Overview of retinoid me-

11. Laudet V, Gronemeyer H.

15. Ko¨nig G, Masters CL, Beyreuther K. Retinoid acid

17. Lane MA, Bailey SJ. Role of retinoid signalling in the

12. Maden M. Retinoid signalling in the develop-

10. Blomhoff R, Blomhoff HK. Overview of retinoid me-

11. Laudet V, Gronemeyer H.

15. Ko¨nig G, Masters CL, Beyreuther K. Retinoid acid

17. Lane MA, Bailey SJ. Role of retinoid signalling in the

12. Maden M. Retinoid signalling in the develop-

10. Blomhoff R, Blomhoff HK. Overview of retinoid me-

11. Laudet V, Gronemeyer H.

15. Ko¨nig G, Masters CL, Beyreuther K. Retinoid acid

17. Lane MA, Bailey SJ. Role of retinoid signalling in the

12. Maden M. Retinoid signalling in the develop-

10. Blomhoff R, Blomhoff HK. Overview of retinoid me-

11. Laudet V, Gronemeyer H.

15. Ko¨nig G, Masters CL, Beyreuther K. Retinoid acid

17. Lane MA, Bailey SJ. Role of retinoid signalling in the