

## Role of retinoid signalling in the adult brain

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### Abstract

Vitamin A (all-*trans*-retinol) is the parent compound of a family of natural and synthetic compounds, the retinoids. Retinoids regulate gene transcription in numerous cells and tissues by binding to nuclear retinoid receptor proteins, which act as transcription factors. Much of the research conducted on retinoid signalling in the nervous system has focussed on developmental effects in the embryonic or early postnatal brain. Here, we review the increasing body of evidence indicating that retinoid signalling plays an important role in the function of the mature brain. Components of the metabolic pathway for retinoids have been identified in adult brain tissues, suggesting that all-*trans*-retinoic acid (ATRA) can be synthesized in discrete regions of the brain. The distribution of retinoid receptor proteins in the adult nervous system is different from that seen during development; and suggests that retinoid signalling is likely to have a physiological role in adult cortex, amygdala, hypothalamus, hippocampus, striatum and associated brain regions. A number of neuronal specific genes contain recognition sequences for the retinoid receptor proteins and can be directly regulated by retinoids. Disruption of retinoid signalling pathways in rodent models indicates their involvement in regulating synaptic plasticity and associated learning and memory behaviours. Retinoid signalling pathways have also been implicated in the pathophysiology of Alzheimer's disease, schizophrenia and depression. Overall, the data underscore the likely importance of adequate nutritional Vitamin A status for adult brain function and highlight retinoid signalling pathways as potential novel therapeutic targets for neurological diseases.

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*Abbreviations:* 9-*cis*-RA, 9-*cis*-retinoic acid; 13-*cis*-RA, 13-*cis*-retinoic acid; AD, Alzheimer's disease; ADH, alcohol dehydrogenase; APP, amyloid protein precursor; ATRA, all-*trans*-retinoic acid; CNS, central nervous system; CRABP, cellular retinoic acid-binding protein; CRBP, cellular retinol binding protein; DHA, docosahexaenoic acid; HATs, histone acetyl transferases; HDACs, histone deacetylase complexes; LTD, long-term depression; LTP, long-term potentiation; RAR, retinoic acid receptor; RXR, retinoid "X" receptor; RARE, retinoic acid response element; RXR, retinoid "X" response element; RALDH, retinal dehydrogenase; ROLDH, retinol dehydrogenase; RBP, retinol binding protein; VAD, Vitamin A deficiency

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## 1. Introduction

Vitamin A and its derivatives, the retinoids, regulate the growth, survival, and differentiation of many cell types. To date, much of the research conducted on Vitamin A and retinoids in the nervous system has focussed on embryonic or early postnatal brain development. It is now well established that retinoids regulate genes which control neuronal differentiation, neurite outgrowth, and the patterning of the anteroposterior axis of the neural tube. These effects likely account for the teratogenic effects of Vitamin A (for recent reviews see Maden, 2002; McCaffery et al., 2003). More recently, evidence has emerged that retinoid signalling may also be required for several aspects of adult brain function (see also Mey and McCaffery, 2004).

We begin this review by outlining the pathways of retinoid metabolism and the localization of components of the retinoid signalling cascade in the adult brain. We then consider the evidence linking retinoid signalling pathways to normal neuronal function and how deficits in retinoid signalling may contribute to the pathology of Alzheimer's disease, schizophrenia and depression. The effects of retinoids are mediated by specific nuclear receptors which act as transcription factors to alter gene expression. However, non-transcriptionally mediated effects of retinoids have also been reported. For example, in the retina retinoic acid acts as a light-signalling neuromodulator and regulates gap junction-mediated coupling of retinal neurons (reviewed by Weiler et al., 2001). Our focus in this review is primarily on distinguishing evidence for retinoid-regulated control of neuronal gene expression in adult neurones, from the retinoid effects on developmental processes of neurogenesis or neurodifferentiation.

This exciting new area of neuroscience research highlights the potential importance of adequate nutritional Vitamin A status for adult brain function. Normally, our nutritional requirements for Vitamin A can be adequately met by a well balanced diet. Dietary excess has only rarely been reported, for example, in the unusual case of toxicity resulting from the consumption of polar bear liver (Rodahl and Moore, 1943). Since retinoids readily enter the central nervous system (Bendich and Langseth, 1989; Snodgrass, 1992), Vitamin A neurotoxicity in adults is possible from excessive consumption of supplements (e.g. Wieland et al.,

1971; Restak, 1972). A prominent symptom of hypervitaminosis A in adults is headaches caused by increased intracranial pressure due to brain swelling resulting in pseudotumour cerebri (Snodgrass, 1992). The mechanism by which hypervitaminosis A causes increased intracranial pressure is unknown but it can eventually lead to coma and death (Macapinlac and Olson, 1981). The incidence of Vitamin A toxicity is relatively rare when compared to the incidence of Vitamin A deficiency (VAD) (Bendich and Langseth, 1989). Worldwide, Vitamin A deficiency is the most common micronutrient deficiency. Very little is known about the cellular effects of VAD or excess exogenous retinoids on the adult human brain. In this review we will summarize the data available, from in vitro and in vivo animal models, which suggest that retinoid signalling pathways are important for adult neuronal function in health and disease.

## 2. Retinoid metabolism in the adult nervous system

Animal-derived foods contain preformed Vitamin A predominantly as retinyl esters. Plant-derived foods contain pro-Vitamin A carotenoids, such as  $\beta$ -carotene. The majority of preformed Vitamin A and pro-Vitamin A carotenoids are converted to all-*trans*-retinol (Vitamin A alcohol) by a series of reactions in both the intestinal lumen and mucosa. Once absorbed by the enterocyte, retinol is re-esterified and packaged with other dietary lipids into chylomicrons for transport to the liver. The liver is the major site of retinoid storage and processing in the body. Retinol is secreted from the liver in response to the body's needs and is transported in the blood bound to retinol binding protein (RBP) (Soprano and Blaner, 1994; Vogel et al., 1999). Circulating retinol is transported to its target tissue bound in a 1:1 ratio to RBP/transthyretin. At most target cells, retinol diffuses freely through the plasma membrane because of its lipophilic nature.

### 2.1. Active retinoids

Once inside the cell, retinol has several potential fates. The predominant pathways of retinol metabolism are summarized in Fig. 1. First, retinol can be stored intracellularly as retinyl esters, providing an immediate

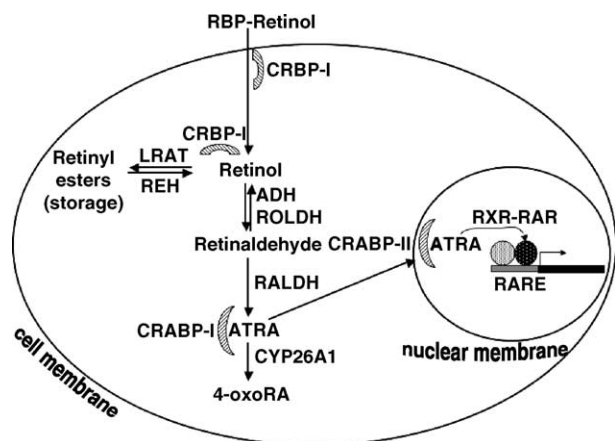


Fig. 1. Intracellular retinol metabolism and retinoid signalling pathways. Retinol is metabolized to all-*trans*-retinoic acid (ATRA) that regulates gene transcription via retinoic acid receptors (RAR) and/or retinoid X receptors (RXRs), which are bound to retinoic acid response elements (RARE). Shaded arcs indicate binding proteins. Other abbreviations: 4-oxoRA, 4-oxoretinoic acid; ADH, alcohol dehydrogenase; CRBP, cellular retinol binding protein; CRABP, cellular retinoic acid-binding protein; LRAT, lethicin:retinol acyltransferase; RBP, retinol binding protein; REH, retinyl ester hydrolase; ROLDH, retinol dehydrogenase; RALDH, retinal dehydrogenase.

store of differentiation-inducing retinoids within the target cell. Second, retinol can be metabolized to its bioactive derivative, all-*trans*-retinoic acid (ATRA). ATRA exerts the most potent effects of any retinoid in a wide variety of biological systems and is therefore the most widely studied Vitamin A derivative.

ATRA affects cellular differentiation and growth by binding to retinoic acid receptors (RAR) and effecting gene transcription (Section 2.3). In addition to the well-studied ATRA/RAR pathway, several relatively new pathways resulting in bioactive retinoids exist. For example, retinol can be metabolized to all-*trans*-4-oxoretinol by the enzyme CYP26A1 in cultured embryonic stem cells (Lane et al., 1999). 4-Oxoretinol can induce gene transcription via RAR (Achkar et al., 1996) in a mechanism similar to ATRA. In addition, retinol can be converted to the retinoids, which can affect cell growth and differentiation exclusive of the retinoid receptors (Buck et al., 1991, 1993; Korichneva and Hammerling, 1999). In the adult brain, these alternative metabolites have not been described.

The biosynthesis of ATRA is due to two sequential oxidations. The first reaction, catalysed by retinol dehydrogenases (ROLDH) or cytosolic alcohol dehydrogenases (ADH), converts retinol to retinal (retinaldehyde). Retinal is then oxidized to ATRA by retinal dehydrogenases (RALDH). The RALDH are members of the class I ALDH family and are also known as ALDH1a1-3 (Vogel et al., 1999). Several forms of each enzyme occur in vivo and are expressed in distinct regions of the adult brain indicating that ATRA synthesis occurs.

ATRA synthesis has been demonstrated in adult rabbit, rat and mouse brain tissue (Dev et al., 1993; Werner and

Deluca, 2002; Wagner et al., 2002). In the cerebrum, cerebellum and meninges, rates of ATRA synthesis were comparable to, or exceeded, rates measured in rat liver (Dev et al., 1993). McCaffery and Drager (1994) demonstrated the presence of RALDH in the pia mater and meninges. By examining the expression of three isoforms of RALDH, Wagner et al. (2002) localized ATRA synthesis to the basal ganglia, hippocampus and auditory afferents in the adult brain. In addition, blood vessels throughout the entire brain expressed RALDH1. RALDH2 was found primarily in the meninges and perivascular cells of the olfactory bulb (Thompson Haskell et al., 2002). However, ATRA can function in a paracrine manner so cells that express RALDH are not necessarily retinoid responsive. A functional effect of local ATRA production on gene expression was suggested by studies using transgenic mice expressing the LacZ gene downstream of three canonical retinoic acid response elements (RAREs) (Thompson Haskell et al., 2002). Transgene expression was found in limbic structures, the dorsal horn of the spinal cord, as well as in the granule cells and periglomerular layers of the olfactory bulb, an area that maintains the capacity for neurogenesis in the adult. These studies demonstrate that the enzymes required for ATRA synthesis are present in discrete areas of the adult brain and that synthesized ATRA can activate gene expression in these same areas.

## 2.2. Retinoid binding proteins

Retinol is transported through the blood stream bound to RBP, but there are also cytosolic RBPs with a high binding affinity for retinoids. Cellular retinol binding proteins (CRBP-I and -II) and cellular retinoic acid-binding proteins (CRABP-I and -II) bind retinol (CRBP-I), retinol and retinal (CRBP-II) and ATRA (CRABP-I and -II), and are members of the fatty acid-binding protein superfamily. CRBP-I is expressed ubiquitously, facilitates retinol uptake into the target cell and directs the intracellular metabolism of retinol to its storage form (retinyl esters) or its active metabolite (ATRA) (Ong et al., 1994; Napoli, 1996; Vogel et al., 1999). In contrast, CRBP-II is expressed only in the villi of enterocytes where it directs the metabolism of retinol to retinyl esters for chylomicron export (Ong et al., 1994). Recently, two novel CRBPs, CRBP-III and IV, each encoded by distinct genes, have been described in the human and mouse, but their functions are not yet understood (Vogel et al., 2001; Folli et al., 2001, 2002).

CRABPs are hypothesized to solubilize and protect ATRA in the aqueous cytosol and to act as shuttles to move lipophilic ATRA to various subcellular components. Both CRABP-I and -II are present in the cytosol and cell nuclei. They are thought to regulate the ability of ATRA to bind to its receptors and thereby alter gene transcription (Delva et al., 1999; Dong et al., 1999; Yamamoto et al., 1998; Zheng et al., 1996). In the adult, CRABP-I is expressed ubiquitously, but CRABP-II is expressed only in the skin,

Table 1  
Location of retinoid binding proteins and receptors in the adult CNS<sup>a</sup>

Protein	Adult location
CRBP-I	Dendritic layers of hippocampus and hippocampal nuclei, dentate gyrus, layer five cortical neurones, walls of cerebral blood vessels, meninges, choroid plexus, ependymal cells, tanocytes, glial elements, neurones in glomerular layer of the olfactory bulb, medial amygdaloid nucleus, and hypothalamic nuclei (Zetterstrom et al., 1994, 1999)
CRABP-I	Striatum, glomerular layer and inner granular layer of the olfactory bulb, olfactory nerve, cells clustered near hypothalamus (Zetterstrom et al., 1994, 1999)
RAR $\alpha$	Olfactory bulb, particularly the glomerular layer, tenia tecta, indusium griseum, cingulate, frontal and parietal cortex, hippocampal fields CA1, 2 and 3, amygdaloid basolateral and lateral nuclei, thalamus, cerebellar lobules, pons, pituitary, choroid plexus and laminae IV–X of the spinal cord (Krezel et al., 1999)
RAR $\beta$	Caudate/putamen and both shell and core of nucleus accumbens, arcuate hypothalamus and dorsomedial hypothalamic nucleus, solitary track and area postrema and rostroventrolateral reticular nucleus of the medulla oblongata, and pituitary gland (Krezel et al., 1999)
RAR $\gamma$	Low levels detected in most diencephalic and rhombencephalic regions especially the hippocampus (Krezel et al., 1999)
RXR $\alpha$	Low levels detected (Krezel et al., 1999)
RXR $\beta$	Ubiquitous distribution but very little protein detected (Krezel et al., 1999)
RXR $\gamma$	Caudate/putamen and shell and core of nucleus accumbens, hypothalamus, pituitary (Krezel et al., 1999)

<sup>a</sup> Table summarizes protein distribution of retinoid binding proteins and receptors. Gene expression data are not included.

uterus, ovary, and choroid plexus, some cholinergic neurones and the pia mater (Dong et al., 1999; Zetterstrom et al., 1999).

Both CRABP-I and -II are present in cell nuclei, where their presumed function is to deliver ATRA to RAR. Kinetic studies of the movement of ATRA to RAR show that CRABP-I is a passive vehicle, binding and releasing its ligand depending on concentration gradients. CRABP-I appears to function to decrease cellular responses to ATRA by catalysing its degradation and thereby lowering active intracellular ATRA concentrations (Fiorella et al., 1993). In contrast, CRABP-II appears to increase ATRA-mediated gene transcription, sensitizing cells to the effects of ATRA. CRABP-II delivers ATRA to RAR in a direct collisional process (Dong et al., 1999; Delva et al., 1999).

The distribution patterns of CRBP-I and CRABP-I and -II in the adult CNS (Table 1) is different from that in embryos and neonates. Developmental changes in the expression of these binding proteins are regulated in a complex spatial and temporal manner as the organism develops, from gastrulation through fetus, which is beyond the scope of this review. In the adult brain, CRBP-I distribution parallels that of RALDH and ATRA with prominent expression in the meninges, the hippocampus, amygdala and the olfactory bulb (Zetterstrom et al., 1999, 1994; McCaffery and Drager, 1994). These data indicate that active metabolism of retinol to ATRA, aided by CRBP-I, is occurring within the adult brain. Interestingly, CRABP-I is also expressed in the hippocampus and olfactory bulb, where it is thought to regulate ATRA concentrations by facilitating the degradation of ATRA to 4-oxoRA, thereby limiting the effects of ATRA in these cells. The expression of CRABP-II in the adult brain is restricted to cholinergic neurones in the basal forebrain and nucleus accumbens and the pia mater. Since retinoid receptor transcripts have not been detected in these areas, CRABP-II may be serving as a retinoid reservoir in these neurones (Zetterstrom et al., 1999).

### 2.3. Retinoid receptors

The actions of retinol's metabolites are primarily mediated by nuclear retinoid receptor proteins termed retinoic acid receptors (RAR- $\alpha$ , - $\beta$ , and - $\gamma$ ) and retinoid "X" receptors (RXR- $\alpha$ , - $\beta$ , and - $\gamma$ ) (Fig. 2). These receptors are members of the steroid receptor family and essentially they function as transcription factors. Each subtype ( $\alpha$ ,  $\beta$ , or  $\gamma$ ) of receptor is encoded by a different gene that has multiple promoters, the products of which can undergo differential splicing, resulting in multiple receptor isoforms (for example, RAR $\beta$ 1, RAR $\beta$ 2, RAR $\beta$ 3 and RAR $\beta$ 4). This complexity gives rise to numerous receptor combinations as RAR exist as heterodimers with RXR; and RXRs can heterodimerize with numerous nuclear receptor proteins.

The RAR and RXR, as with all nuclear receptors, contain a DNA-binding and ligand-binding domains (Fig. 2B). The ligand specificities of these receptors differ. RAR bind ATRA with high affinity and 9-*cis*-retinoic acid (9-*cis*-RA) with lower affinity, whereas RXR bind 9-*cis*-RA (Soprano et al., 2004). The effects of Vitamin A and its derivatives on gene transcription are presumed to be mediated largely by ATRA interactions with RAR since 9-*cis*-RA is undetectable in any tissues in vivo (Mic et al., 2003). Activation of the RAR–RXR heterodimer is controlled by RAR because RXR-selective ligands do not induce gene transcription, a phenomenon termed "RAR dominance" (Kurokawa et al., 1994). However, once an RAR ligand is bound, RXR ligands can increase the transcriptional efficiency of the RAR–RXR heterodimer (Minucci et al., 1997).

Recent studies suggest that although RAR are concentrated in cell nuclei, they can shuttle between the nucleus and cytoplasm like other nuclear receptors. For example, chimeras consisting of RAR and glucocorticoid receptors have been shown to shuttle between the cytoplasm and nucleus (Mackem et al., 2001). Similarly, Maruvada et al. (2003) demonstrated that approximately 20% of RAR-green fluorescent protein (GFP) chimeras were cytosolic but

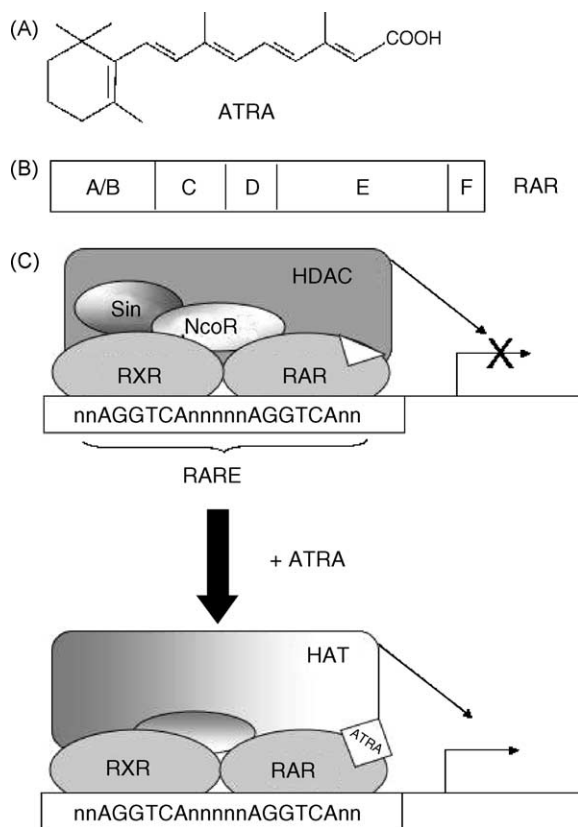


Fig. 2. all-*trans*-Retinoic acid (ATRA) regulates gene transcription via retinoic acid receptors and/or retinoid X receptors (RAR–RXR) bound to retinoic acid response elements (RARE). (A) Chemical structure of ATRA. (B) Diagram of RAR structure to illustrate the multiple functional domains. The A/B domain contains ligand-independent transactivation activity. The C domain contains two zinc fingers responsible for binding to RARE. The D domain contains the hinge region. ATRA binds to the E domain, which also contains ligand-dependent transactivation activity. The function of the F domain is unknown. RXR have a similar functional organization but do not bind ATRA (Soprano et al., 2004). (C) RAR and RXR bind to RAREs. Unliganded RARs are complexed with histone deacetylase complexes (HDAC), inhibiting gene transcription. Upon ATRA binding, the HDAC and coregulator proteins are released and new proteins, with histone acetyl transferase (HAT) activity, are recruited.

moved rapidly to the nucleus on binding ATRA. The intracellular distribution of RAR in adult neurons has not been examined. However, the trafficking of ligand bound RAR to the nucleus where it can induce gene expression represents a new way in which RAR, and other nuclear receptors, can affect cellular function.

The distinct temporal and spatial expression pattern of each retinoid receptor subtype and isoform suggests that they each may regulate specific neuronal phenomena (Table 1). Only two of the RAR, RAR $\alpha$  and RAR $\beta$ , are detected at high levels in the adult brain with RAR $\gamma$  expression being very low. RAR $\alpha$  protein has a fairly widespread distribution with particularly high levels in the hippocampus and cortex (Krezel et al., 1999). On the other hand, RAR $\beta$  and RXR $\gamma$  have a more restricted distribution including dopamine-innervated areas such as the caudate/putamen and nucleus accumbens (Krezel et al., 1999). The

distribution of the RAR indicates the potential for several areas of the adult brain to respond to retinoids. Indeed, studies performed in isoform specific RAR and RXR null double mutant mice demonstrate that this is the case (Sections 4 and 5). The mRNA distribution of the RARs, RXRs, and CRABP-II has been examined (Zetterstrom et al., 1999); however, for clarity, we do not include it in this discussion here because the actual proteins must be present for retinoid signalling to occur.

Unliganded RXRs are co-factors for non-steroid nuclear receptors including thyroid hormone receptors and the Vitamin D receptor (Bugge et al., 1992; Kliewer et al., 1992). When RXR exogenous agonists are used, RXR function as ligand-activated heterodimeric binding partners for numerous nuclear receptor proteins including PPAR $\gamma$ , LXR, FXR and the orphan receptor nur77/NGFB-1 (Perlmann and Jansson, 1995; Kliewer et al., 1995; Janowski et al., 1996; Forman et al., 1995). Interestingly, RXR can bind to the brain-enriched fatty acid docosahexaenoic acid (DHA) (Mangelsdorf and Evans, 1995; Mata de Urquiza et al., 1999). DHA has been shown to be neuroprotective and to increase the survival of dopaminergic neurones (Glozman et al., 1998; Lauritzen et al., 2000; Politi et al., 2001). This increase in survival was blocked by RXR antagonists (Wallen-Mackenzie et al., 2003). In addition, RXR are binding partners for the orphan nuclear receptor, Nurr1, which is expressed almost exclusively in the CNS and is essential for the development of midbrain dopaminergic neurones (Castillo et al., 1998; Le et al., 1999; Saucedo-Cardenas et al., 1998; Wallen et al., 1999; Wallen-Mackenzie et al., 2003). The observation that DHA activates the transcriptional activity of Nurr1–RXR heterodimers in the developing CNS (Wallen-Mackenzie et al., 2003) indicates that DHA–RXR interactions may also play a functional role in the maintenance of mature neurones.

#### 2.4. Retinoic acid response elements

To affect gene transcription, ATRA must bind to RAR (Fig. 2C). The RARs exist as heterodimers with RXRs and bind to RAREs, while RXRs can homodimerize and bind to RXREs (retinoid X responsive elements or retinoid responsive elements) (Soprano et al., 2004). RAREs are usually composed of direct repeats of the consensus half-site sequence AGGTCA separated by five nucleotides (Fig. 2C). A less common form of RARE, termed a DR-2 RARE, is found in the promoter regions of some genes, for example CRBP-I (Mangelsdorf et al., 1991). The arrangement of, and space between, the RARE half-sites affects receptor specificity and binding. RXREs are generally direct repeats of the same consensus sequence separated by only one nucleotide. Although direct repeats are common, the half-sites can also be arranged as palindromes or inverted palindromes. The RXR portion of the RAR–RXR heterodimer binds to the 5' upstream half-site with the RAR

Table 2  
Retinoid-regulated neuronal genes<sup>a</sup>

Gene	Effect of RA on expression	Cell system	Assay	References
<b>Transporters</b>				
Norepinephrine transporter	↑	PC12, SCG	mRNA, function	Matsuoka et al. (1997)
Vesicular ACh transporter	↑	PC12, SN56, NG108	mRNA	Berse and Blusztajn (1995, 1997) and Dolezal et al. (2001)
Vesicular GABA transporter	↑	P19	mRNA	Ebihara et al. (2003)
<b>Metabolic enzymes</b>				
Choline acetyltransferase <sup>P</sup>	↑	PC12, SN56 NG108-15, SCG	mRNA, protein, activity	Berse and Blusztajn (1995), Berse and Blusztajn (1997) and Personett et al. (2000) Dolezal et al. (2001), Kobayashi et al. (1994) and Pedersen et al. (1995)
Tyrosine hydroxylase	↓	SCG	mRNA, activity	Kobayashi et al. (1994), Berrard et al. (1993) and Cervini et al. (1994)
Dopamine β-hydroxylase	↓	SCG	Activity	Berrard et al. (1993) and Cervini et al. (1994)
Glutamic acid decarboxylase	↑	P19, NE-7C2	mRNA	Bain et al. (1993) and Varju et al. (2002)
ACh esterase	↑	P19	mRNA	Coleman and Taylor (1996)
<b>G-protein coupled receptors</b>				
Mu opioid receptor	↓/↑	SH-SY5Y	mRNA	Jenab and Inturrisi (2002)
Delta opioid receptor	↑	NG108-15	mRNA	Beczowska et al. (1996)
Kappa opioid receptor	↓	P19	mRNA	Bi et al. (2001) and Hu et al. (2002)
5-HT <sub>1A</sub> receptor	↑	SN-48	mRNA	Charest et al. (1993)
Dopamine D2 receptor <sup>V</sup>	↑	Striatum	mRNA, protein	Samad et al. (1997) and Valdenaire et al. (1998)
Dopamine D2, D1, D5 receptors	↑	NT2	mRNA, function	Sodja et al. (2002)
<b>Ionotropic receptors</b>				
Nicotinic AChR α3,α4,β2	↑	P19	mRNA, protein	Cauley et al. (1996)
NMDA receptor (NR1)	↑	NG108-15	mRNA	Beczowska et al. (1996)
Kainate receptor (GluR6)	↑	P19	mRNA	Bain et al. (1996)
GABA <sub>A</sub> receptor γ2	↑	P19	mRNA, function	Reynolds et al. (1996)
<b>Ion transport proteins</b>				
K <sup>+</sup> channel (Kir 2.1)	↑	SH-SY5Y, SK-N-BE	mRNA, function	Smith-Maxwell et al. (1991), Arcangeli et al. (1998) and Tonini et al. (1999)
Na <sup>+</sup> /H <sup>+</sup> exchanger	↑	P19	mRNA, activity	Dyck and Fliegel (1995)
L-type Ca <sup>2+</sup> channel	↑	NG108-15, NT2	mRNA, function	Kamp et al. (1995) and Gao et al. (1998)
N-type Ca <sup>2+</sup> channel	↑	NT2	mRNA, function	Gao et al. (1998)
<b>Cytoskeletal proteins</b>				
Neurofilament (-L,-M,-H) proteins	↑	P19	mRNA, protein	Chiu et al. (1995) and Paterno et al. (1997)
Dystrophin-associated proteins	↑	P19	mRNA	Ceccarini et al. (2002)
Tenascin	↑	SH-SY5Y	Protein	Linnala et al. (1997)
Integrin α/β	↑	SH-SY5Y	Protein	Linnala et al. (1997)
<b>Intracellular signalling molecules</b>				
Phospholipase A2, C, D	↑	LA-N-1	Activity	Farooqui et al. (2004)
Arachidonic acid	↑	SK-N-BE	Activity	Petroni et al. (1996a, 1996b)
Neuronal nitric oxide synthase	↑	SN56	mRNA, protein, activity	Personett et al. (2000)
Tissue transglutaminase	↑	SCG, SK-N-BE, SH-SY5Y	Activity, mRNA	Ando et al. (1996), Melino et al. (1997) and Tucholski and Johnson (2003)
Neuromodulin (GAP 43)	↑	Striatum		Husson et al. (2004)
Neurogranin (RC3) <sup>V</sup>	↑	SK-N-BE, striatum	mRNA	Husson et al. (2004) and Iniguez et al. (1994)

Table 2 (Continued)

Gene	Effect of RA on expression	Cell system	Assay	References
Neuron-specific enolase <sup>P</sup>	↑	N-115, PC12	mRNA	Matranga et al. (1993) and Sakimura et al. (1995)
Calbindin-D 28K	↑	PC12, D283	mRNA, protein	Vyas et al. (1994) and Wang and Christakos (1995)
Synapsins I, II, III	↑	NT2	mRNA	Leypoldt et al. (2002)
Miscellaneous				
Gonadotropin-releasing hormone <sup>V</sup>	↓/↑	GT1-1, hypothalamus	mRNA	Cho et al. (2001a, 2001b, 1998)
Oxytocin <sup>V</sup>	↑	Neuro2A	mRNA	Richard and Zingg (1991)
Trk A/Trk B	↑	NB, P19	mRNA	Lucarelli et al. (1995) and Salvatore et al. (1995)
Nerve growth factor	↑	PC12	mRNA	Scheibe and Wagner (1992)
Neural cell adhesion molecule	↑	P19, LA-N-5	Protein	Husmann et al. (1989) and Cervello et al. (1997)
Presenilin 1	↑	SH-SY5Y	mRNA	Flood et al. (2004)
Presenilin 2	↑	P19	mRNA	Culvenor et al. (2000)
Apolipoprotein E	↓	NT2	mRNA	Harris et al. (2004)
Amyloid protein precursor (APP) <sup>P</sup>	↑	P19, SH-SY5Y, PC12, hippocampus	mRNA, protein	Hung et al. (1992), Fukuchi et al. (1992), König et al. (1990), Lahiri and Nall (1995) and Yang et al. (1998)
Microtubule-associated protein tau (MAPT) <sup>P</sup>	↑	P19	mRNA	Fukuchi et al. (1992) and Heicklen-Klein et al. (2000)

<sup>a</sup> Table summarizes data which show that retinoic acid (RA) can increase (↑) or decrease (↓) the expression of specific neuronal genes as assayed by mRNA, protein or functional activity in a variety of neuronal cell lines (PC12 rat pheochromocytoma cells; SCG rat superior cervical ganglia neurons; SN56 clonal cholinergic cell line derived from medial septum of mouse forebrain; SN-48 a murine septum × neuroblastoma fusion cell line; SH-SY5Y human neuroblastoma cell line; GT-1 mouse-derived hypothalamic GnRH neuronal cells; P19 embryonal carcinoma cells; NG108-15 mouse neuroblastoma X rat glioma hybrid cell line; LA-N-1/5 human neuroblastoma cell line; SK-N-BE human neuroblastoma cell line; N-115 mouse neuroblastoma cell line; N2AB-1 mouse neuroblastoma cell line; NB human neuroblastoma; D283 human medulloblastoma cell line; NT2 human teratocarcinoma cell line; Ntera 2/C1.D1 neuroblastoma; NE-7C2 mouse p53-deficient neuroectodermal cell-line). The superscripts P and V refer to a putative or verified RARE in the promoter of the gene concerned.

occupying the downstream half-site (Fig. 2C) (Piedrafita and Pfahl, 1999).

RAREs have been identified in the regulatory regions of several genes involved in retinoid signalling that are expressed in the adult brain including RAR $\alpha$ 2, RAR $\beta$ 2, RAR $\gamma$ 2, CRBP-I, and CRABP-II (Piedrafita and Pfahl, 1999). The identification of genes containing active RARE is difficult because genes regulated by retinoids, for example neurogranin (Husson et al., 2004), may be a secondary target of a primary protein regulated by ATRA, and may not themselves contain a RARE. However, RAREs have been identified in a number of neuronal genes which further supports the notion that retinoid-mediated regulation of gene transcription may be important in the function of the adult brain (Table 2, Section 3).

Some RAREs are also bound by receptors other than RAR and RXR, such as the COUP-TF receptor and the orphan nuclear receptors TOR and Tak-1 (Hirose et al., 1995; Ortiz et al., 1995; Tran et al., 1992). COUP-TF1 and COUP-TFII (ARP-1) are also expressed in distinct patterns in the adult brain. Particularly high levels of COUP-TF1 expression are found in the olfactory nucleus, neocortex, dentate gyrus, hippocampus and the granular layer of the cerebellum (Lopes de Silva et al., 1995). In contrast, COUP-TFII is expressed in the midbrain with high levels of

expression in reticular and thalamic nuclei, as well as the arachnoid membranes (Lopes de Silva et al., 1995). Retinoid binding proteins and receptors are also expressed in several of these areas; however, we do not know if they are expressed in the same cells as the COUP-TFs (Table 1). These COUP-TF receptors bind to the RARE and block the binding of RAR–RXR heterodimers to the DNA, preventing retinoid-induced gene transcription (Piedrafita and Pfahl, 1999). The presence of COUP-TF proteins in the adult brain indicates that they may compete with RAR and RXR to regulate retinoid-mediated neuronal gene expression, although this has not been tested.

Retinoids can also inhibit gene transcription through a RARE-independent mechanism, involving AP-1. This mechanism is not well understood but ATRA-bound RAR–RXR heterodimers appear to repress AP-1 activity (Li et al., 1996; Salbert et al., 1993; Schule et al., 1991; Yang-Yen et al., 1991). Additionally, the AP-1 component proteins, Fos and Jun, down regulate the retinoid signal when present at high levels (Pfahl, 1993). AP-1 regulates numerous genes that tend to increase cellular proliferation. In contrast, retinoids generally inhibit cellular proliferation and induce differentiation. Therefore, it is hypothesized that the interaction between AP1 and RAR–RXR switches cells from a proliferative to a differentiated phenotype (Piedrafita and Pfahl, 1999). The

role of AP-1 in repressing retinoid-mediated gene expression in the adult brain has not been examined yet.

### 2.5. Co-regulators

In the absence of ATRA, a corepressor is bound to the RAR, preventing gene transcription via RAREs. When ATRA binds the RAR, the corepressor is released and replaced with a coactivator, resulting in the induction of gene transcription. These corepressors and coactivators mediate their effects by recruiting proteins that moderate the acetylation of histones surrounding the DNA containing the RARE. Thus, RAR corepressor proteins recruit histone deacetylase complexes (HDACs) to remove the acetyl groups from the lysine residues of histones and prevent gene transcription. In contrast, RAR coactivator proteins recruit histone acetyl transferases (HATs) to acetylate these lysine residues, unwind DNA and facilitate gene transcription (Fig. 2C) (reviewed by Wei, 2003).

The major RAR corepressors are nuclear receptor corepressor (N-CoR) and SMRT (silencing mediator for retinoid and thyroid hormone receptors) (Chen and Evans, 1995; Piedrafita and Pfahl, 1999). These receptors do not interact with ligand bound RAR, but bind, along with a Sin3, to unliganded RAR and recruit HDACs to repress transcription (Fig. 2C). Upon ATRA binding, the ligand-binding domain of the RAR changes shape and releases the corepressors. Coactivators are then recruited to the RAR. Coactivators may enhance the basal transcriptional machinery or, like N-CoR, recruit additional proteins such as phosphoCREB-binding protein which display histone acetylase activity, freeing the DNA from the histones and allowing transcription to occur (Piedrafita and Pfahl, 1999). As with AP-1, the role of coactivators and corepressors of retinoid-mediated gene transcription has yet to be examined in the adult brain.

### 3. Neuronal targets of retinoid-regulated gene expression

We have surveyed the literature for evidence that neuronal specific genes can be regulated by retinoids (Table 2). By and large, the evidence reported here is taken from *in vitro* cell systems that are models of neuronal differentiation. Many are pluripotent cells that under the influence of ATRA differentiate to a neuronal phenotype. For the purposes of this review, genes involved in neuronal differentiation and maturation pathways, or those that are part of the retinoid signalling cascade (RAR, CRBP, CRABP, etc.), have not been included. Rather, we have chosen to focus on the large number of neuronal specific genes identified in such systems. Genes from all walks of neuronal life are potential downstream targets of retinoid-mediated transcriptional regulation in adults. Interestingly, individual neurotransmitter systems may be regulatable by retinoids at several different levels. For example, retinoids can upregulate the expression of glutamic acid decarbox-

ylase (involved in the synthesis of GABA), the GABA transporter and GABA<sub>A</sub> receptor  $\gamma 2$  subunit mRNAs (Table 2). Such multi-layered regulation complicates the potential effects of retinoid administration in the adult brain.

To what extent these genes are directly transcriptionally controlled by ATRA in the adult brain remains to be tested. In Table 2, putative (P) RAREs are indicated where ATRA has been shown to have a direct effect on promoter constructs such as choline acetyltransferase and neurone specific enolase. Verified (V) RAREs are indicated where sequence analysis and detailed promoter studies have confirmed a direct interaction between RA and RAREs, such as in the 5' UTR of the dopamine D2 receptor (Samad et al., 1997; Valdenaire et al., 1998), gonadotropin-releasing hormone (Cho et al., 2001a, 2001b), oxytocin (Richard and Zingg, 1991) and neurogranin (Iniguez et al., 1994). Importantly, the verified oxytocin RARE is not apparently regulated in hypothalamic neurons, but is active in peripheral tissues such as the uterus (Larcher et al., 1995). This means that even for neuronal genes with verified RARE it is not straightforward to predict whether they will be sensitive to retinoid regulation in the adult brain. For most of the genes listed, the existence of an RARE in the promoter region has not been examined and therefore it is impossible to tell whether such genes are capable of being directly regulated by retinoids in the adult central nervous system. Interestingly, the expression of most of these genes is upregulated in response to RA, whereas tyrosine hydroxylase and dopamine  $\beta$  hydroxylase are downregulated in sympathetic neurones cultured in the presence of ATRA (Berrard et al., 1993; Cervini et al., 1994; Kobayashi et al., 1994). This is consistent with the switch from noradrenergic to cholinergic phenotype in these neurones. It remains unclear whether such regulation is a direct ATRA-mediated transcriptional effect or a secondary consequence of differentiation to a cholinergic phenotype. Alternative mechanisms for ATRA-mediated regulation of mRNA expression have been suggested. ATRA can apparently increase the expression of some mRNA species by increasing mRNA stability, e.g., calbindin-D28 (Wang and Christakos, 1995) and acetylcholine esterase (Coleman and Taylor, 1996).

Clearly much more work is needed in differentiated neuronal cells and in adult *in vivo* systems to establish whether any of these genes can be directly regulated in the adult nervous system. Furthermore, detailed promoter analyses should identify whether verifiable RAREs are present in more neuronal genes. The available data on the functional effects of RA-regulated gene expression in adult neuronal systems are discussed below (Sections 4–6).

### 4. Physiological role of retinoids in hippocampal function

A role for ATRA in the physiological function of the hippocampus is suggested by the presence of components of

retinoid signalling pathways (Table 1). RALDH2 protein, one of the enzymes that synthesizes RA, is restricted to the meninges surrounding the hippocampus in the adult mouse brain (Wagner et al., 2002). Immunoreactivity for the binding proteins CRBP-I and CRABP-I immunoreactivity has been demonstrated in the dendritic layers of the hippocampal formation and the dentate gyrus (Zetterstrom et al., 1994). In addition, RAR $\alpha$  and RXR $\alpha$ ,  $\beta$ ,  $\gamma$  mRNA transcripts are detected in the hippocampus (Zetterstrom et al., 1999). Given that the hippocampus is viewed as playing a central role in memory and spatial learning, it is perhaps not surprising that retinoid signalling has been shown to have an effect on these processes.

#### 4.1. Synaptic plasticity

Long-lasting, activity-dependent changes in synaptic efficacy are thought to be the cellular mechanisms underlying memory and learning (Bliss and Collingridge, 1993). ATRA signalling has been implicated in changes in both adult long-term potentiation (LTP) and long-term depression (LTD) of synaptic function in the hippocampus.

Impaired LTP and LTD have been demonstrated in mice lacking the receptors for RAR $\beta$  alone, or both RAR $\beta$  and RAR $\gamma$  (Chiang et al., 1998). Although RAR $\alpha$  is abundantly expressed in the adult hippocampus, targeted disruption of RAR $\alpha$  in mice results in early postnatal lethality (Lufkin et al., 1993). In the CA1 region of the hippocampus LTP was essentially absent in RAR $\beta$  or RAR $\beta$ -RXR $\gamma$  null mutant mice. This is perhaps surprising since RAR $\beta$  is not reported to be expressed in hippocampus (Table 2) and indicates an indirect effect of RAR $\beta$  is involved. Interestingly, LTP remained intact in mice deficient only in RAR $\gamma$  suggesting a specific involvement of RAR $\beta$  containing receptors in regulating LTP. In addition, LTD could not be induced in RAR $\beta$  or RAR $\beta$ -RXR $\gamma$  or RXR $\gamma$  mutant mice. As ATRA alters gene expression in the developing brain via RARs, the LTP and LTD impairment observed could have been interpreted as a developmental deficit. However, electron microscopy revealed that there were no ultrastructural abnormalities or gross abnormalities in synaptic function in these mice (Chiang et al., 1998).

The importance of retinoid signalling in synaptic plasticity is supported by observations in Vitamin A deficient (VAD) mice. In adult mice deprived of Vitamin A for 12 weeks, both LTP and LTD are reduced, while LTD was completely abolished after 15 weeks (Misner et al., 2001). Both phenomena could be reversed after administration of a Vitamin A supplemented diet clearly indicating that synaptic plasticity is regulatable by retinoids in adult mice. Furthermore, LTP is significantly reduced in aged mice compared with younger adults and this impairment can be partially reversed by treatment with RA (Etchamendy et al., 2001). This partial reversal highlights that retinoid regulation is only one component underlying age-related deficits in LTP.

These findings suggest that while synaptic responses per se are not affected, synaptic plasticity is regulated to some extent by retinoid signalling. Retinoids are potent regulators of transcription and the cellular mechanisms underpinning LTP and LTD are thought to involve changes in gene expression (Pittenger and Kandel, 1998). So what are the downstream targets that could be involved in retinoid regulation of learning and memory? VAD mice exhibit a hypoexpression of RAR $\beta$  and RAR $\beta$ / $\gamma$  reversible by ATRA, in the hippocampus compared to Vitamin A sufficient control animals (Etchamendy et al., 2003), indicating that ATRA-mediated gene expression would be impaired in this model. Neurogranin (RC3) and neuromodulin (GAP43) have been proposed to play an important role in synaptic plasticity by regulating calcium availability and calcium/calmodulin-dependent protein kinases (Gerendasy and Sutcliffe, 1997; Krucker et al., 2002). In vitro studies indicate that neurogranin expression is upregulated by ATRA (Iniguez et al., 1994). Prolonged VAD in mice resulted in a significant decrease in neurogranin (RC3) expression that could not be reversed by ATRA (Etchamendy et al., 2003). In the hippocampus of VAD rats, there is a reduced expression of RXR $\beta$ / $\gamma$  but no change in either neurogranin or neuromodulin (GAP43) expression (Husson et al., 2004). Species differences or age differences may account for the differences reported in these VAD models, but neurogranin remains a potentially interesting target of retinoid signalling. In addition, there may be direct effects of ATRA on the expression of ionotropic glutamate receptors (Bain et al., 1996; Beczkowska et al., 1996). However, the precise involvement of specific genes in RA-mediated regulation of synaptic plasticity has not been determined.

#### 4.2. Learning and memory

Transgenic mouse models where RARs have been deleted, Vitamin A deficiency and aging mice have all been used to study the effects of retinoids on learning and memory behaviours. The performance of RAR $\beta$  or RAR $\beta$ -RXR $\gamma$  but not RXR $\gamma$ , null mutant mice in the Morris Water Maze task revealed deficits in spatial learning and memory (Chiang et al., 1998). In the radial maze spatial learning task, rats fed on a Vitamin A deficient diet for 12 weeks made more errors than controls, an effect that could be reversed by replenishing Vitamin A in the diet for about 2 weeks (Cocco et al., 2002). On the other hand, in mice, VAD diets needed to be maintained for 28 weeks before any symptoms of Vitamin A deficiency could be observed (Etchamendy et al., 2003). Deficits in spatial learning were evident in VAD mice treated for 39 weeks (but not 31 weeks) and the effect was not reversed by daily administration of ATRA (150  $\mu$ g/kg) for 10 days (Etchamendy et al., 2003). These two studies may highlight differences between species or more likely methodological difficulties in achieving an equivalent VAD state.

VAD is very difficult to induce in mouse, but less so in rats, because mice pups are born with sufficient Vitamin A stores to last till their entire lives (McCaffery et al., 2003). Therefore, it is generally accepted that to induce VAD postnatally, the dam must be fed a VAD diet while pregnant. However, this approach inevitably leads to developmental deficits that complicate the study of retinoid regulation of adult pathways. Consequently, to study the effects of Vitamin A on adult animals, a state of Vitamin A “deprivation” rather than a true deficiency is used (Cocco et al., 2002; Etchamendy et al., 2003; Misner et al., 2001). Indeed, dietary Vitamin A deprivation for 10 weeks in adult rats results in serum retinal and liver retinol concentrations that were reduced to 70% of control (Husson et al., 2003).

Aging mice provide an alternative model in which to study the effects of ATRA signalling on learning and memory. In 21–23-month-old animals, compared with younger adult mice (4–5 months), a relational memory deficit was apparent that could be reversed by treatment with ATRA (150 µg/kg) for 10 days (Etchamendy et al., 2001). Again, no evidence was reported to confirm the VAD status or circulating retinoid levels in these experiments. Rather the authors used evidence that the expression of RARs and target genes are reduced in aged animals. Furthermore, these age-related reductions are susceptible to reversal by acute RA treatment (Enderlin et al., 1997), and the reversal effect of RA could be blocked by coadministration of the RAR antagonist CD3106 (Etchamendy et al., 2001). Interestingly, another retinoid, 13-*cis*-RA (Accutane, Section 6.3), can induce impairment in the ability of mice to perform the radial arm maze task (Crandall et al., 2004). This effect has been attributed to reduced adult hippocampal neurogenesis (Section 4.3).

Clearly there is a link between retinoid signalling and synaptic plasticity, as well as learning and memory behaviours. Taking all the data together, a possible pathway for retinoid-mediated effects on synaptic plasticity and learning behaviour emerges. In the absence of Vitamin A or reduced ATRA signalling, activation of RAR is reduced, which may lead to reduced expression of neurogranin and possibly other important neuronal genes. Reduced availability of neurogranin, or reduced expression of other neuronal genes, in VAD rats or mice would alter the calmodulin availability but the downstream cellular effects in terms of calmodulin availability or CaMKII phosphorylation have not been tested. However, impairments in hippocampal synaptic plasticity do correlate with the impaired spatial learning deficits described when retinoids are manipulated.

#### 4.3. Adult neurogenesis

Given the role of retinoids in regulating neuronal differentiation during development (Maden, 2002), it is perhaps not surprising that retinoids have also been shown to regulate adult neurogenesis. The phenomenon of adult

neurogenesis, where new neurones proliferate and become functionally integrated with existing neurones, has been most widely studied in the hippocampus (Kempermann et al., 2004). Since ATRA synthesis has been localized to the hippocampus and RALDH2 is present in the meninges, there is an endogenous source of RA to regulate this process (Wagner et al., 2002; Thompson Haskell et al., 2002). The adult hippocampus contains multipotent progenitor cells, found in the subgranular zone, that can generate both glia and neurones in vitro (Palmer et al., 1997). *all-trans*-RA enhances the ability of these progenitor cells to differentiate to a neuronal phenotype, although neurotrophins are also required to promote neuronal maturation (Takahashi et al., 1999). In contrast, long-term retinoid treatment in vivo has the opposite effect on adult neurogenesis (Crandall et al., 2004). When mice were treated with 13-*cis*-RA (1 mg/kg) for up to 6 weeks, a significant decrease in hippocampal neurogenesis was observed (Section 6.3). Since adult neurogenesis is thought to contribute to processes involved in learning and memory, these authors also tested learning behaviour in the radial maze task. 13-*cis*-RA treated mice made significantly more errors than controls indicating that there was a cognitive impairment (Crandall et al., 2004). This chronic, long-term retinoid treatment may also have effects on synaptic plasticity (Section 4.1) and neuronal gene expression, which may contribute to the altered spatial learning behaviour.

### 5. Physiological role of retinoids in dopaminergic systems

Dopaminergic neurones are found principally in the midbrain (substantia nigra and ventral tegmental area) and the major dopaminergic pathways project from the midbrain to the striatum, the frontal cortex and the limbic system. The distribution of dopaminergic pathways is consistent with the multiple roles ascribed to dopamine including coordination of movement, reward and emotional processing. Interestingly, key components of retinoid signalling, including RALDH, RARs and RA binding proteins, are found in the dopaminergic pathways (Table 1). In addition, in neuronal cells RA regulates the expression and activity of tyrosine hydroxylase and dopamine β hydroxylase (the enzymes that synthesize dopamine) and the dopamine D2 receptor (Table 2). Such observations have given rise to the idea that retinoid signalling may be an important influence on dopaminergic function and dopaminergic signalling in the adult brain.

#### 5.1. Locomotion

In retinoid receptor knockout mice, a locomotor deficit is evident that is accompanied by decreased dopamine D2 receptor expression. In RARβ-RXRβ, RARβ-RXRγ and RXRβ-RXRγ double null mutant mice (but not the

corresponding single mutants) reduced forward locomotion and rearing frequency was observed in the open field test (Krezel et al., 1998). Motor coordination was tested by performance on the rotarod and the same double null mutant mice quickly fell indicating impaired performance. Mutants involving either RAR $\alpha$  or RAR $\gamma$  alone, or the RAR $\alpha$ –RXR $\gamma$  combination, did not show any defects in locomotor tests, suggesting a specificity of the retinoid signalling pathways, with respect to RAR $\beta$ , RXR $\beta$  and RXR $\gamma$ , is involved in regulating movement. In addition to the locomotor deficit, the expression of dopamine D1 and D2 receptors was decreased 40% in the striatum of RAR $\beta$ –RXR $\beta$ , RAR $\beta$ –RXR $\gamma$  and RXR $\beta$ –RXR $\gamma$  null mice, but not in RAR $\beta$  or RXR $\gamma$  single mutants (Krezel et al., 1998). This confirmed an earlier report by Samad et al. (1997) that RXR $\beta$ –RXR $\gamma$  and RAR $\alpha$ –RXR $\gamma$  double mutant mice had a 40% reduction in D2 receptor mRNA expression. Although these authors suggested that the single RXR $\gamma$  mutant mice also had a reduced expression of D2 receptors. Whatever the specific role of the RARs, these *in vivo* results are consistent with observations in a rat pituitary cell line that ATRA can directly regulate dopamine D2 receptor expression via an identified RARE in its promoter (Valdenaire et al., 1994, 1998).

RA mediated effects have been implicated in Parkinson's disease (Eichele, 1997) and Huntington's disease (Luthi-Carter et al., 2000) on the basis of an interaction with striatal dopamine D2 receptors. Null mutation of the D2 receptor in mice results in altered locomotor behaviours that are consistent with basal ganglia dysfunction and a Parkinsonian-like phenotype (Fowler et al., 2002). Similarly, a study of a transgenic mouse model for Huntington's disease used gene expression profiling of the striatum to identify changes in gene expression (Luthi-Carter et al., 2000). At least 20% of the numerous genes with reduced expression contained RAREs and included RXR $\gamma$ , RBP and the D2 receptor. Such reports are indicative of an involvement of RA pathways in the pathology of Parkinson's or Huntington's disease but further work is required to substantiate this.

The use of RAR–RXR transgenic mice means that a neurodevelopmental effect cannot be excluded, even though gross histological abnormalities could not be detected (Krezel et al., 1996, 1998). Compensatory changes in receptor expression could occur during development that may produce subtle differences in the mature neuronal function. For example, NURR1 is an orphan nuclear receptor expressed developmentally and in the adult central nervous system that can heterodimerize with RXR, suggesting that NURR1 is important for signalling in response to RXR ligands (Aarnisalo et al., 2002; Sacchetti et al., 2001). Furthermore, NURR1 is essential for the differentiation of the midbrain dopamine neurones (Zetterstrom et al., 1997) and heterozygous null mutant mice have altered locomotor behaviours (Eells et al., 2002; Backman et al., 2003). However, these actions of NURR1 on maturation and maintenance of dopamine neurones are

independent of any retinoid effect (Castro et al., 2001; Sacchetti et al., 2001; Hermanson et al., 2003), consistent with the general view that retinoid-based RXR ligands do not exist *in vivo*. Rather the transcriptional regulation via RXR may be regulated by DHA (Section 2.4).

The expression of other dopaminergic markers, such as tyrosine hydroxylase expression, has not been reported in RAR–RXR mutant mice. Such data might indicate whether or not there was a developmental deficit of dopaminergic signalling in transgenic mice. Interestingly, in VAD adult mice and rats locomotor deficits or decreased abundance of D2 mRNA have not been reported. In the absence of such evidence, it is difficult to conclude whether the locomotor deficit seen in RAR–RXR mutant mice arises when retinoid signalling pathways are compromised during development or whether the expression of the dopamine D2 receptor is mis-regulated in the adult brain and this directly leads to a locomotor deficit.

## 6. Retinoid pathways and disease

### 6.1. Alzheimer's

Retinoid signalling pathways have been implicated in the pathology of Alzheimer's disease (AD) (Goodman and Pardee, 2003). Clinically, AD is characterized by progressive memory impairment and deteriorating cognitive ability. The involvement of RA in cognitive functions, such as learning and memory, has been reported (Section 4.2). The disease is also defined by the formation of amyloid plaques, the presence of neurofibrillary tangles, and ultimately neuronal loss. Familial AD has been linked to mutations or altered function of a number of genes including those involved in the formation of amyloid plaques (Bertoli-Avella et al., 2004). Interestingly, several of these amyloid plaque genes are regulated by retinoid signalling *in vitro* (Table 2). Furthermore, genetic linkage studies have shown an association of chromosomes 10q23 and 12q13 (Myers and Goate, 2001) with Alzheimer's disease. These loci include genes of the retinoid signalling cascade such as RBP4 and RAR $\gamma$  (Goodman and Pardee, 2003).

The cascade of events leading to amyloid plaque formation begins with proteolytic cleavage of the amyloid precursor protein (APP) to form amyloid  $\beta$  protein. The presenilins (PS1 and PS2) form part of the multiprotein complex  $\gamma$ -secretase that regulates the cleavage of APP. Application of ATRA to cells induces an upregulation of APP mRNA (Konig et al., 1990; Fukuchi et al., 1992) that is likely mediated by a direct effect on the APP promoter; although a definitive RARE has not yet been reported (Lahiri and Nall, 1995; Yang et al., 1998). PS1 and PS2 are also upregulated by ATRA *in vitro*, an effect which might promote plaque formation (Hong et al., 1999; Culvenor et al., 2000; Flood et al., 2004). Indeed increased expression of PS2 has been reported in AD and mutations in both PS1

and PS2 are reported in early-onset familial AD (Zekowski et al., 2004). Apart from effects on plaque formation, overexpression of PS1/2 may predispose towards AD via actions on calcium homeostasis and apoptotic pathways.

Recently, the effects of retinoid signalling on amyloid plaque formation have been directly tested. Ono et al. (2004) demonstrated that Vitamin A had anti-amyloidogenic and de-stabilizing effects on fibril formation in vitro. This effect was independent of any changes in gene expression and likely resulted from anti-oxidant properties of Vitamin A (Ono et al., 2004). This is consistent with observations in a VAD rat model (Corcoran et al., 2004). One-year-old rats, deprived of Vitamin A since weaning, were immunopositive for amyloid  $\beta$  protein in the cerebral cortex. Furthermore, in aged rats (22–23 months) treated with ATRA daily for 10 days (64 or 640  $\mu\text{g}/\text{kg}$ ), APP mRNA expression in the hippocampus was reduced (Pan et al., 1993). ATRA also altered the relative proportions of different splice variants of APP mRNA, while having no effect on choline acetyltransferase, neurotrophin-3 or nerve growth factor expression. These observations indicate that ATRA might protect against plaque formation mechanisms.

Deficits in cholinergic neurotransmission have been identified as the principal underlying cause of the cognitive deficits in Alzheimer's disease. Cocco et al. (2002) examined acetylcholine synaptic function in VAD rats. Basal acetylcholine release was not different in VAD rats when compared to controls, although scopolamine (a compound which blocks the presynaptic acetylcholine autoreceptor) evoked release was impaired. These findings suggested that acetylcholine release was impaired during the performance of the memory task in VAD rats. Interestingly, in neuronal cell lines, ATRA has been shown to be capable of upregulating the expression and activity of the enzyme that catalyses the synthesis of acetylcholine, choline acetyltransferase (ChAT) most likely via a direct interaction with the ChAT promoter (Kobayashi et al., 1994; Berse and Blusztajn, 1995, 1997; Pedersen et al., 1995; Personett et al., 2000; Dolezal et al., 2001). One possible explanation for the impaired acetylcholine release seen in VAD animals is impaired acetylcholine synthesis. However, the authors did not examine ChAT gene expression in VAD rats (Cocco et al., 2002). A more recent study has demonstrated that the number of ChAT-positive neurones is significantly lower in the forebrain of adult VAD rats than in controls (Corcoran et al., 2004). On the other hand, Pan et al. (1993) found that ATRA had no effect on ChAT mRNA abundance in the hippocampus of adult rats. In addition, acetylcholine esterase mRNA is also upregulated by ATRA. Since this enzyme catabolizes acetylcholine, and cholinesterase inhibitors are used for the treatment of Alzheimer's (Cummings, 2004), it is difficult to see how upregulating the expression of cholinesterase would be beneficial in AD. Overall, it seems that retinoid signalling may have a role in regulating both the synthesis of acetylcholine (via ChAT) and the availability of acetylcholine at synapses (via AChE)

which may play an important role in the pathology of AD, but this requires further investigation.

Apolipoprotein E (ApoE) is the major apolipoprotein in the cerebrospinal fluid and has been identified as a major susceptibility gene in Alzheimer's disease (Bertoli-Avella et al., 2004). Apart from being involved in the transport of retinylesters (Blomhoff, 1994), ApoE has neurotrophic and neuroprotective properties (Gutman et al., 1997; Sun et al., 1998). In neuronal cells the abundance of constitutively expressed ApoE is decreased following ATRA treatment (Harris et al., 2004) while in rat primary astrocyte cultures, ATRA increased the secretion of ApoE (Cedazo-Minguez et al., 2001). The abundance or secretion of ApoE following retinoid manipulation in vivo has not been reported, so it is difficult to draw any conclusions about the relevance of ATRA-mediated regulation of ApoE in AD.

Retinoid signalling has been suggested as a potentially exploitable pathway for devising novel therapies in AD (Goodman and Pardee, 2003). It is difficult to know whether Alzheimer's patients have a deficit in RA signalling since there are conflicting reports in the literature. For example, Rinaldi et al. (2003) reported that plasma Vitamin A levels are reduced in AD, whereas Connor and Sidell (1997) have shown that hippocampal retinoid content is similar in AD and control groups. Corcoran et al. (2004) reported a decreased abundance of RAR $\alpha$  and RALDH2 in AD brains suggesting that RA signalling is likely to be compromised in patients. Studies in aged mice (Section 4.1) indicate that RAR abundance is reduced which may ultimately lead to a decrease in RA-mediated effects. Indeed the cognitive impairments seen in aged rodents can be reversed by pharmacological activation of RA signalling (Etchamendy et al., 2001). However, the pathology of AD is complex involving numerous cellular processes, and retinoids can alter these processes by affecting gene expression as well as acting as an antioxidant. Caution is needed in interpreting individual data as "the retinoid effect in AD". In order to consider retinoid therapy for AD, it is necessary to establish in vivo, in the adult brain, the precise role of Vitamin A and its receptors in the multiple processes involved in regulating plaque formation, neurofibrillary tangles, cholinergic transmission and ApoE function.

## 6.2. Schizophrenia

Schizophrenia is a mental illness that is among the world's top 10 causes of long-term disability. The range of schizophrenic disorders is characterized by symptoms that include psychosis, apathy and withdrawal, and cognitive impairment. Both genetic and environmental factors are thought to play a role in the development of schizophrenia (Mueser and McGurk, 2004). The characteristic changes in brain volume and altered morphology reported in schizophrenia have led to a view of schizophrenia as a neurodevelopmental disorder (Waddington, 1993). Regions such as the frontal lobes, amygdala, hippocampus, para-

hippocampus, thalamus, and medial temporal lobe, cingulate gyrus, and superior temporal gyrus have decreased volumes in patients with schizophrenia compared with controls (Lawrie and Abukmeil, 1998). Such observations suggest that there may be genetic loci associated with altered development of the nervous system. Given the prominent role of retinoids in such processes, it is perhaps not surprising that mapping of several human chromosomal regions associated with susceptibility to schizophrenia has shown a high coincidence of genes involved in the retinoid signalling cascade at these loci. In this review we are primarily focussed on the role of retinoid signalling in the adult brain and the reader is referred to reviews by Goodman (1998) and La Mantia (1999) for more details on the role of retinoids and the neurodevelopmental aspects of schizophrenia.

Retinoid analogues have been proposed as pharmacotherapy for schizophrenia in the adult brain (Goodman, 1998; Citver et al., 2002). Schizophrenia is a complex disorder and multiple neurotransmitter systems have been implicated. Several lines of evidence support the role of altered dopaminergic function in the development of schizophrenia (Davis et al., 1991). Relevant targets of retinoid regulation include dopamine D2 receptor and tyrosine hydroxylase and dopamine  $\beta$ -hydroxylase (Table 2). Antipsychotics that are beneficial for the treatment of schizophrenia are antagonists of the dopamine D2 receptor. The D2 receptor promoter demonstrates a functional polymorphism that is significantly associated with schizophrenia. Samad et al. (1997) have shown that this region contains a functional RARE/RXRE DNA motif that regulates the expression of the D2 receptor. It is difficult to predict how useful a retinoid therapy might be in schizophrenia. Dopamine agonists exacerbate the positive symptoms of schizophrenia (Davis et al., 1991), so a reduced expression of the dopamine synthetic enzymes by retinoids might impair dopamine availability and thereby improve the disease condition. However, typical antipsychotic drugs have strong antagonistic property for dopamine D2 receptors (Davis et al., 1991), so retinoid therapy that increased expression of D2 receptors would not necessarily have a beneficial effect in schizophrenia. Furthermore, alternatively spliced isoforms of the D2 receptor are located pre- and postsynaptically (Centonze et al., 2004), and it is not yet known whether these isoforms are differentially regulated by retinoids. Clearly, further in vivo studies in adult models are required to understand what the overall effects of manipulating retinoid signalling on striatal dopaminergic systems might be.

Although we know that antipsychotic drugs interact with membrane neurotransmitter receptors, such as the dopamine D2 receptors, subsequent biochemical intracellular events triggered by this interaction are not well understood. Recently, antipsychotic drug administration has been shown to have a modest effect on inducing the expression of RAR and RXR in the striatum (Langlois et al., 2001; Ethier et al.,

2004). Chronic treatment of adult rats with haloperidol, but not clozapine, resulted in a small but significant increase in mRNA abundance of RAR $\beta$  and RXR $\gamma$  (the predominant isoforms expressed in the striatum). These data suggest that retinoid-mediated transcriptional regulation may play a role in the long-term response to these drugs. The downstream targets of antipsychotic-induced alterations in retinoid signalling pathways have not yet been identified.

### 6.3. Depression

Accutane (isotretinoin or 13-*cis*-RA) is a synthetic retinoid and an effective oral treatment for severe nodular acne. Between 1982 and 2002 the FDA received reports of over 3000 psychiatric events associated with the use of Accutane, including depression and suicide (Hull and D'Arcy, 2003). Interestingly, the psychiatric effects of hypervitaminosis A have been known for some time, and include increased depressive indices (Restak, 1972; Wieland et al., 1971). However, the possible association of Accutane with depression remains controversial (Jacobs et al., 2001; Wysowski et al., 2001; O'Connell et al., 2003). Existing psychiatric conditions and the association of depression with acne complicates the interpretation of such reports, but there is evidence that psychiatric symptoms recover when Accutane therapy is stopped.

13-*cis*-RA is rapidly isomerized to ATRA within sebocytes (Tsukada et al., 2000) and consequently its actions are attributed to ATRA-induced changes in gene transcription. Both 13-*cis*-RA and ATRA are detected in the serum of patients administered Accutane. 13-*cis*-RA exhibits a low binding efficiency for RAR and does not bind RXR (Tsukada et al., 2000; Idres et al., 2002), although when 13-*cis*-RA does bind to RAR, it is very efficient at activating gene transcription via RARE (Idres et al., 2002). Therefore, both 13-*cis*-RA and RA itself may have actions on the adult nervous system which could account for the reported side effects of Accutane use.

Potential targets for retinoid signalling in depression include dopaminergic, serotonergic or noradrenergic pathways or a complex interaction between these neurotransmitter systems. Regulation of the dopamine D2 receptor by antidepressant drugs has recently been demonstrated. Desipramine, citalopram and mianserin all up-regulate the transcriptional activity of the D2 receptor gene promoter in vitro (Dziedzicka-Wasylewska and Solich, 2004). It is perhaps surprising that these drugs have a common effect on D2 receptor expression, given that these drugs all have very different sites of action: desipramine is a selective noradrenaline reuptake inhibitor, citalopram belongs to the group of selective serotonin reuptake inhibitors, while mianserin is regarded as an antagonist of pre-synaptic  $\alpha_2$ -adrenergic receptors. However, this is an interesting finding suggesting that upregulation of D2 receptors may contribute to antidepressant effects. Since retinoid signalling is required for normal expression of D2 receptors, it is

possible that 13-*cis*-RA has an effect on D2 receptors but this has not been tested.

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) appears to play a key role in the pathophysiology of depression. Altered serotonergic function in depression has been demonstrated in patients displaying lower plasma levels of the serotonin precursor tryptophan, reduced cerebrospinal fluid levels of the catabolic compound 5-hydroxyindolacetic, decreased serotonin uptake and decreased expression of the serotonin transporter and 5-HT<sub>1A</sub> receptor (Neumeister et al., 2004). ATRA has been reported to regulate the differentiation of serotonergic neurones in mouse neural crest cells, to promote the proliferation of these neurones (Ito and Morita, 1995) and to induce the expression of 5-HT<sub>1A</sub> receptors in neuronal cells (Charest et al., 1993). Although these are likely to be developmental effects, it is possible that retinoids could promote these effects *in vivo* in adults.

Recently, the antidepressant effects of the selective serotonin reuptake inhibitor fluoxetine have been shown to require hippocampal adult neurogenesis, mediated via an interaction with 5-HT<sub>1A</sub> receptors (Santarelli et al., 2003). Interestingly, whereas ATRA has been shown to promote adult neurogenesis *in vitro* (Palmer et al., 1997; Takahashi et al., 1999), chronic 13-*cis*-RA administration *in vivo* reveals a significant decrease in hippocampal adult neurogenesis (Crandall et al., 2004) (Section 4.3). Since there are no molecular studies investigating the cellular effects of either ATRA or 13-*cis*-RA on adult neuronal systems involved in depression, it is difficult to reconcile the existing data with the reported side effects of Accutane treatment.

## 7. Summary

Clearly, retinoid signalling has a physiological role in synaptic plasticity and learning and memory behaviours. Vitamin A deprivation in adult mice and rats highlight the importance of adequate Vitamin A status for such cognitive functions; but the precise targets of retinoid signalling pathways underlying these altered behaviours remain to be identified.

Given the large number of neuronal genes that could potentially be transcriptionally regulated by retinoids in the adult brain, surprisingly little is known about the impact of ATRA signalling on other brain functions. The use of transgenic mouse models has indicated a role for retinoids in the regulation of striatal dopaminergic function and the control of locomotor activity. However, such models do not clearly delineate between the well-described developmental effects of retinoids and any novel adult-specific effects.

Future research needs to be conducted in this emerging field to deepen our understanding of the role retinoids play in the adult brain. Retinoids, retinoid agonists and antagonists offer great potential as therapeutics in schizophrenia,

depression, Alzheimer's and possibly even Parkinson's or Huntington's diseases. However, before such treatments could be pursued we need to know more about the neuronal genes and cellular processes that are specifically regulated by retinoids and the functional consequences in the adult brain.

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