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Distribution of the Vitamin D receptor and 1α-hydroxylase in human brain

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Abstract

Despite a growing body of evidence that Vitamin D is involved in mammalian brain functioning, there has been a lack of direct evidence about its role in the human brain. This paper reports, for the first time, the distribution of the 1,25-dihydroxyvitamin D_3 receptor (VDR), and 1α-hydroxylase (1α-OHase), the enzyme responsible for the formation of the active vitamin in the human brain. The receptor and the enzyme were found in both neurons and glial cells in a regional and layer-specific pattern. The VDR was restricted to the nucleus whilst 1α-OHase was distributed throughout the cytoplasm. The distribution of the VDR in human brain was strikingly similar to that reported in rodents. Many regions contained equivalent amounts of both the VDR and 1α-OHase, however the macrocellular cells within the nucleus basalis of Meynert (NBM) and the Purkinje cells in the cerebellum expressed 1α-OHase in the absence of VDR. The strongest immunohistochemical staining for both the receptor and enzyme was in the hypothalamus and in the large (presumably dopaminergic) neurons within the substantia nigra. The observed distribution of the VDR is consistent with the proposal that Vitamin D operates in a similar fashion to the known neurosteroids. The widespread distribution of 1α-OHase and the VDR suggests that Vitamin D may have autocrine/paracrine properties in the human brain.

Keywords: Vitamin D; Neurosteroids; 1α-hydroxylase; Human brain; Brain development

1. Introduction

The active form of Vitamin D, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) is a pluripotent seco-steroid with physiological functions that extend beyond its classical role in calcium homeostasis and bone metabolism (Jones et al., 1998).With respect to this, several recent studies have postulated functions for $1,25(OH)_2D_3$ in both in the developing (Eyles et al., 2003) and adult brain (McGrath et al., 2001; Garcion et al., 2002). In particular our group has postulated that low levels of Vitamin D during early life may be relevant to several brain diseases such as schizophrenia and multiple sclerosis (McGrath, 2001). Although expression of the receptor for $1,25(OH)_2D_3$ (Vitamin D receptor, VDR) has been described in the brains of several species, evidence for the presence of the receptor in human brain has been scant.

Previous studies have confirmed that the VDR is expressed in both developing (Veenstra et al., 1998a; Burkert et al., 2003) and adult rat brain (Stumpf and O'Brien, 1987; Clemens et al., 1988; Prufer et al., 1999) as well as in adult hamster brain (Musiol et al., 1992). The only previous investigation reporting VDR expression in human brain was by Sutherland et al. (1992) via in situ hybridization. Using radiolabeled cDNA probes, these researchers showed that VDR mRNA is expressed in the brains of patients with Alzheimer's or Huntington's disease. The VDR has also been shown to be expressed in a human

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neuroblastoma cell line (Moore et al., 1996). Evidence for VDR protein in human brain is still lacking.

Given the lack of data concerning the distribution of VDR in non-diseased human brain, we performed an immunohistochemical study in order to map the distribution of the VDR in multiple brain regions. The presence of a functional receptor however would mean little if the ligand could not actually gain access to the brain. The early autoradiographic studies would appear to indicate that this ligand partitions widely throughout the brain but this evidence is equivocal (Carswell, 1997). An alternative mechanism is that $1,25(OH)_2D_3$ may be synthesized locally within the brain via expression of the enzyme 1α-hydroxylase (1α-OHase) which catalyzes conversion of $25OHD_3$ to $1,25(OH)_2D_3$. The enzyme is classically expressed in the kidney (Zehnder and Hewison, 1999) but immunohistochemical techniques have been used to describe the distribution of this enzyme in non-renal human tissues as well (Zehnder et al., 2001). In the latter study only two regions of the brain were examined but nevertheless strong expression of 1α-OHase was observed in the cerebellar Purkinje cells as well as within neuronal cells of the cerebral cortex, thus indicating that local production of the active vitamin within human brain was possible. 1α-OHase has also been detected in foetal human brain (Fu et al., 1997). In addition, 1α-OHase has been shown previously to be both present within glial cells in culture and capable of the final hydroxylation of 25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D_3 (Neveu et al., 1994b). We have therefore elected to examine multiple regions of the human brain for this enzyme and compare these with the distribution for the VDR.

The aims of this study are threefold; firstly to confirm the presence of the VDR and 1α-OHase in human brain; secondly to map their distribution in selected brain regions

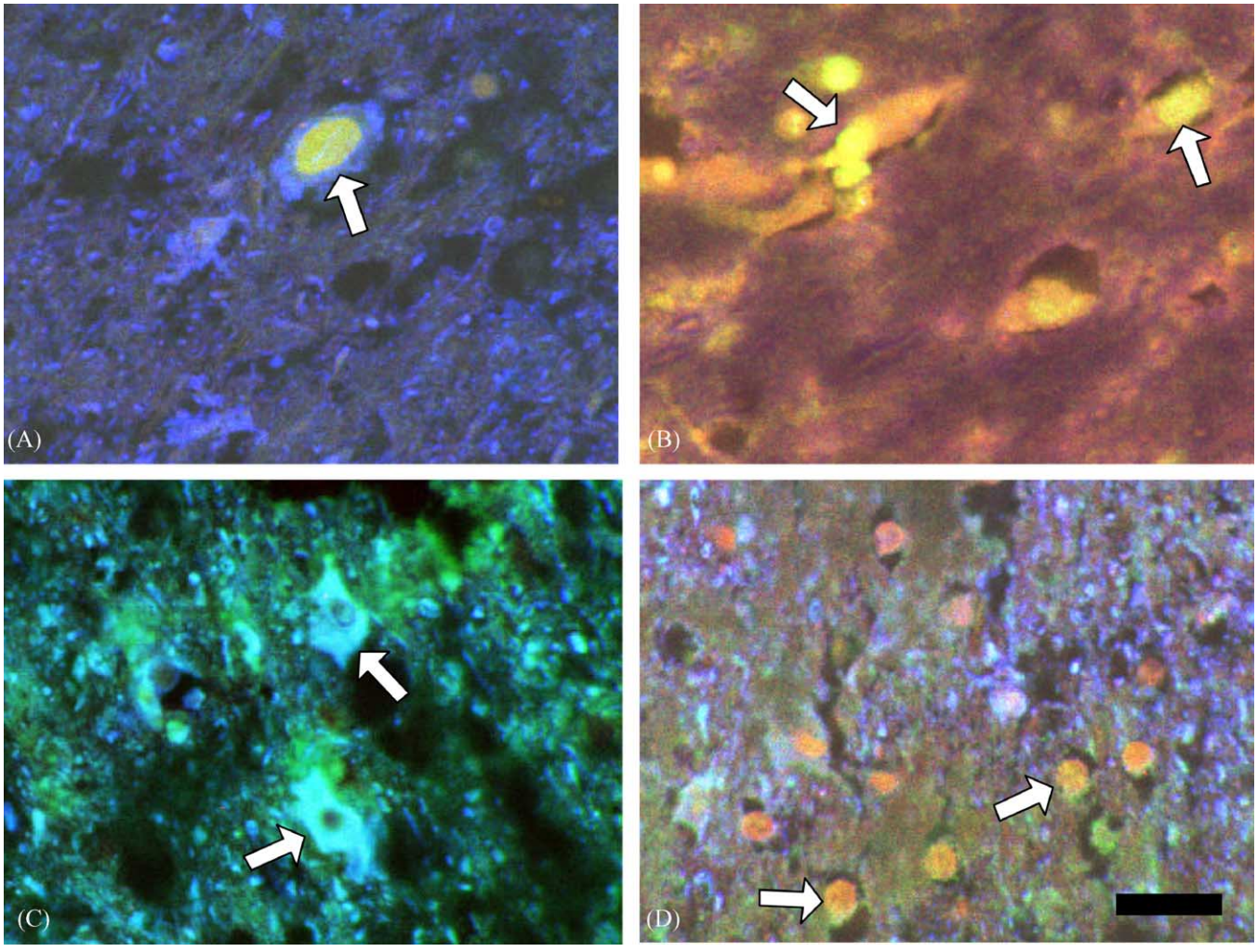

Fig. 1. Immunofluorescent detection of VDR and 1α-OH in both neurons and glia. (A) Neurofilament 200-Cy5 labeled neuron (arrow) displaying the FITC labeled VDR within the nucleus. (B) GFAP-Cy3 labeled cells also show VDR (FITC) immunoreactivity within the nucleus (arrows). (C) Neurofilament 200-Cy5 labeled neurons also express 1α-OH (FITC) within the cytoplasm producing a pale blue colour (arrows). (D) Glia labeled with GFAP-Cy3 also express 1α-OH-FITC resulting in orange colourization (arrows). Bar = 20 μm.

and thirdly to determine if cells expressing either the receptor and/or the enzyme are neuronal or glial in origin.

2. Materials and methods

2.1. Immunohistochemistry

Human brain tissue was obtained from the New South Wales tissue resource centre, Sydney, Australia. Formalin fixed brain tissue was selected from five males (34–58 years) all from individuals free of any psychopathology or history of alcohol or drug abuse. Tissue had been stored between 15 and 36 months in 10% formalin (sodium phosphate buffered, pH 7.0). Post-mortem intervals ranged from 12 to 25 h. Tissue segments were transferred to 0.32 M sucrose in 0.1 M phosphate buffer for 7 days before being placed in 1:1 solution of 30% sucrose and OCT embedding media (Sakura, Tokyo, Japan) for 12 h before embedding in 100% OCT. Frozen 42 μM coronal sections were obtained on an IEC Minotome Plus cryostat and kept in 0.1 M Tris buffer (pH 7.4) containing 0.1% sodium azide at 4 °C until required. Free-floating sections were antigen retrieved in 0.01 M sodium citrate buffer (pH 6.0) by heating until boiling in a microwave oven. Endogenous peroxidase activity was quenched by 30 min exposure to 1% hydrogen peroxide in 0.05 M Tris buffered saline (TBS, pH 7.6). Sections were then incubated in 10% horse serum in TBS for 45 min to prevent non-specific antibody binding. After rinsing in TBS (3 × 15 min) the sections were exposed to primary antibodies diluted in blocking solution (1:500) for one hour at room temperature. Primary antibodies consisted of a monoclonal antibody to VDR (Chemicon, CA) or a polyclonal antibody to sheep 1α-OHase (The Binding Site, Birmingham, UK). Following further washes in TBS, sections were incubated with secondary antibodies (1:200 in TBS + 10% horse serum) consisting of either a biotinylated anti-mouse IgG (VDR), or a biotinylated anti-sheep IgG (1α-OHase) (Vector Laboratories Inc., CA) for 45 min at room temperature. The sections were washed three times in TBS before labeling of bound antibodies was enhanced by exposing the sections to a preformed avidin and biotinylated horseradish peroxidase macromolecular complex as per manufacturer's directions (Vector Laboratories Inc., CA). Following three washes in TBS, antibodies were visualized using diaminobenzidine (Sigma, MO) and sections were mounted on glass slides and coverslipped with Permount. Given a slightly less defined cellular staining for this antigen sections stained for 1α-OHase were lightly counterstained with cresyl violet prior to mounting to help define cell structure. For all nuclei an adjacent section was stained with haemotoxylin and eosin (H and E) from all five subjects. This not only helped to orientate the particular brain nuclei examined but also aided in assigning a qualitative score for the percentage of cells immunostained within that region. Staining intensity was rated by two observers (DE and SS) with >95% agreement on score assignment.

2.2. Immunofluorescence

Sections were antigen retrieved as described above before incubation with a blocking solution consisting of 5% horse serum and 5% BSA in TBS for 45 min. Neurons were labeled with rabbit anti-neurofilament 200 (1:150; Sigma, MO) diluted in TBS containing 1% BSA and 0.3% Triton X-100 incubated overnight at 4 °C. Following washes in TBS, sections were incubated with an anti-rabbit secondary antibody conjugated to Cy 5 (1:200; Zymed, CA) for 2 h at room temperature. To detect glia, sections were exposed to monoclonal anti-glial-fibrillary acidic protein (GFAP) conjugated to Cy 3 (1:200; Sigma, MO). Immunological

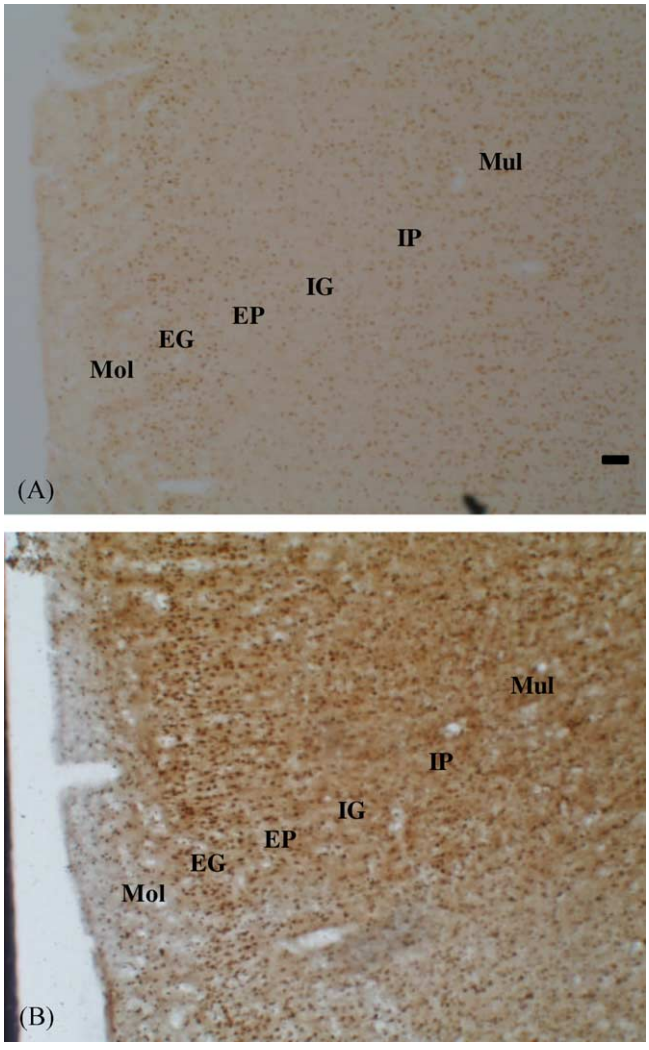

Fig. 2. (A) Cells within the prefrontal cortex stain for VDR, in particular note external granule layer staining. (B) 1α-OH immunoreactivity is present in most cells of the prefrontal cortex, with more intense staining found in the external granule layer than in deeper layers. Mol: molecular layer, EG: external granule layer, EP: external pyramidal, IG: internal granule, IP: internal pyramidal, Mul: multiform layer. Bar = 100 μm.

detection of VDR and 1α-OHase was as previously described except that a fluorescein anti-mouse IgG and a fluorescein anti-sheep IgG conjugate (1:200; Sigma, MO) were used as secondary antibodies for anti-VDR and anti-1α-OHase, respectively. Before mounting in 75% glycerol, sections were stained with 1% Sudan black to mask lipofuscin induced autofluorescence (Schnell et al., 1999). Sections were viewed with a Radiance2000 Confocal (BioRad, CA) coupled to a Nikon E600 microscope and images recorded using Lasersharp2000 software (BioRad, CA).

3. Results

3.1. General observations

The pattern and intensity of immunohistochemical responses to both antigens were similar in all five post-mortem samples. VDR immunoreactivity was present in both neurons and glia. Its sub-cellular distribution was strictly nuclear (Fig. 1A and B). Staining intensity for VDR varied widely throughout the brain. In general white matter regions were poorly immunoreactive. 1α-OHase was abundant in all brain regions examined. Like VDR its immunoreactivity was present in both neurons and glia. However in contrast to VDR, staining was restricted only to the cytoplasm (Fig. 1C and D). Weak immunoreactivity was also seen in the white matter adjacent to the regions examined. With respect to the VDR, comparison with H and E stained sections showed that most cells within strongly staining regions were positive for the VDR. In contrast, 1α-OHase staining was far more variable with many (often smaller) cells negative for 1α-OHase even within strongly stained regions. The most notable exception to this was found in the substantia nigra where far fewer large cells stained for VDR compared with 1α-OHase.

3.2. Cortical regions

VDR and 1α-OHase staining was dispersed throughout the two cortical regions investigated. In the prefrontal cortex

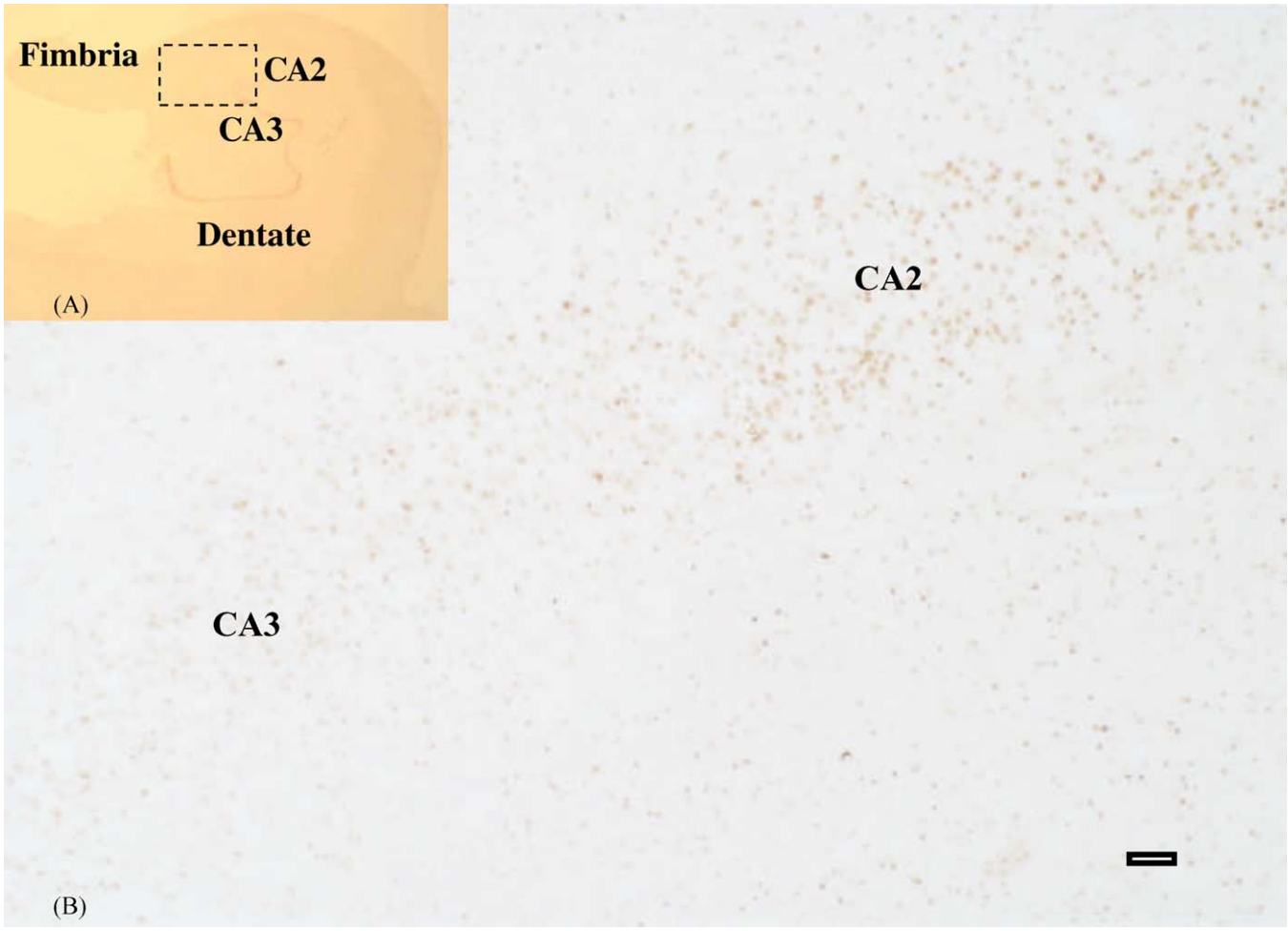

Fig. 3. (A) Low magnification view of section containing hippocampal region immunostained for VDR. (B) Higher magnification view of boxed area of 3A containing the CA2–CA3 junction and showing the discrepancy in VDR immunoreactivity between the pyramidal layers of the CA2 and CA3 regions. Bar = 100 μm.

the most intense VDR immunoreactivity was observed in the external granule layer whilst cells within the molecular layer of the prefrontal cortex were poorly stained (Fig. 2A). 1α-OHase was more prevalent in the molecular layer but again intense staining was present in the external granule layer (Fig. 2B). Granule cells appeared more intensely stained than pyramidal cells.

In the cingulate gyrus a more intense VDR immunoreactivity was found in the molecular layer with moderately intense staining observed in the deeper laminae. Again more intense immunoreactivity was seen in granule cells compared to pyramidal cells. Moderate 1α-OHase immunoreactivity was found within the molecular layer of the cingulate gyrus however, contrary to the prefrontal cortex, no delineation in the degree of immunoreactivity between superficially placed cells and those of deeper layers was apparent. Many non-1α-OHase containing cells were also dispersed throughout the granule and pyramidal layers.

3.3. Allocortex

The pyramidal layer within CA1 and CA2 was strongly immunoreactive for VDR while the cells of this layer within CA3 were not as intensely labeled (Fig. 3). Within the dentate gyrus, granule cells were more heavily stained compared to cells of the molecular and polymorphic layers. In contrast to VDR, 1α-OHase was evenly distributed throughout the CA1, CA2 and CA3 regions of the hippocampus. Again staining was most noticeable in pyramidal cells where all cells appeared stained in contrast to the molecular and polymorphic layers where many cells were unstained. Strong 1α-OHase immunostaining was seen in the granule layer of the dentate gyrus while the molecular and polymorphic layers contained a combination of moderately immunoreactive cells and cells which were devoid of 1α-OHase immunostaining.

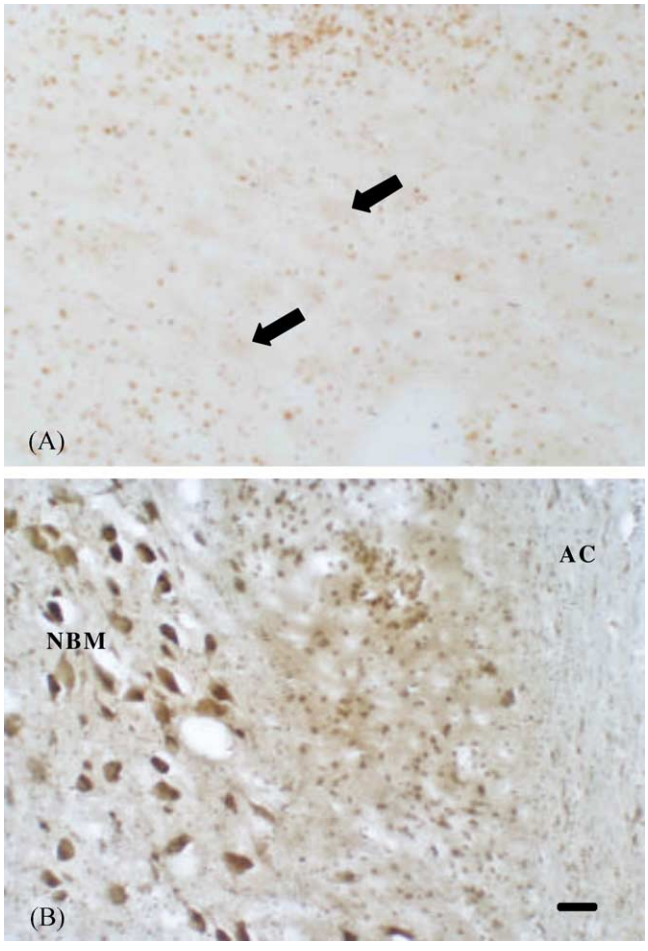

Fig. 4. (A) No VDR immunoreactivity is seen in the macrocellular CH4 neurons within the nucleus basalis of Meynert (NBM) (arrows). (B) Intense staining for 1α-OHase is found within these same neurons. AC: anterior commissure. Bar = 50 μm.

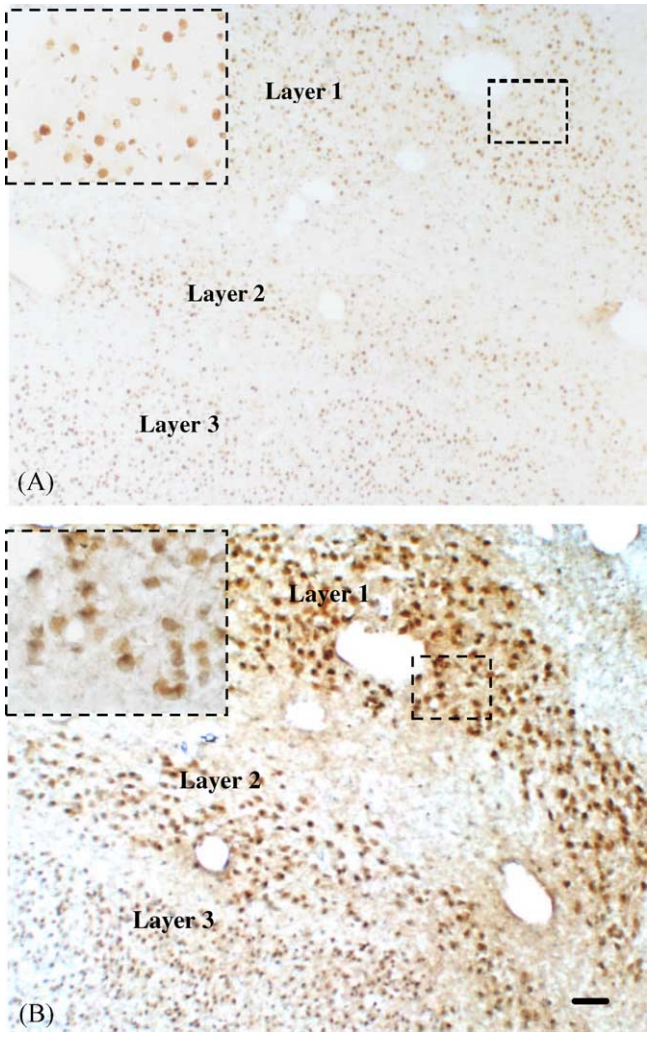

Fig. 5. (A) Strong VDR immunoreactivity is found within all layers of the lateral geniculate nucleus. (B) Intense1α-OHase staining is also found within the lateral geniculate. Insets contain magnified view of boxed areas showing the nuclear specificity of VDR staining and the cytoplasmic nature of 1α-OH immunoreactivity. Bar = 100 μm.

3.4. Non-cortical regions

Macrocellular cells within both the nucleus basalis of Meynert (NBM) and septum conspicuously did not appear to express VDR protein. However these same neurons all appeared to be strongly immunoreactive for 1α-OHase (Fig. 4). VDR staining appeared more intense than that for 1α-OHase in caudate and the putamen. 1α-OHase staining in caudate and putamen was heterogeneous with staining in larger cells appearing to be more intense. Weak staining was found in the internal and external capsules.

All regions within the amygdala displayed poor VDR and only moderate 1α-OHase immunoreactivity both in terms of cell number and staining intensity. No differences were apparent among the corticomedial, central or basolateral groups of nuclei. Many cells of the uncus and entorhinal cortex displayed intense 1α-OHase staining though these regions also contained a number of cells that displayed counterstaining only.

All thalamic nuclei exhibited poor to moderate staining for both VDR and 1α-OHase. Most of the smaller cells displayed little 1α-OHase staining. In contrast the lateral geniculate nucleus displayed strong cellular immunoreactivity for both VDR and 1α-OHase particularly in layer 1 (Fig. 5). Staining for both VDR and 1α-OHase was intense within the large neurons in the substantia nigra. About half the macrocellular neurons within the substantia nigra stained for VDR compared with a much greater proportion of neurons that stained positive for 1α-OHase (Fig. 6).

Both VDR and 1α-OHase displayed intense immunoreactivity in the supraoptic and paraventricular nuclei of the hypothalamus especially in the larger cells. Other regions of this structure contained cells that stained either at a moderate or strong intensity. Many of the smaller cells within the hypothalamus were not immunopositive for 1α-OHase.

The distribution of VDR and 1α-OHase showed stark differences within the cerebellum. VDR was absent from both Purkinje cells and the molecular layer within the cerebellum whilst granule cells displayed strong immunoreactivity (Fig. 7A). In contrast, cells within the molecular layer and all Purkinje cells were moderately immunoreactive for 1α-OHase (Fig. 7B). The granule layer displayed some intense staining but a large portion of this cell population exhibited either weak or no immunoreactivity.

The distribution of both the receptor and the enzyme is summarised in Table 1.

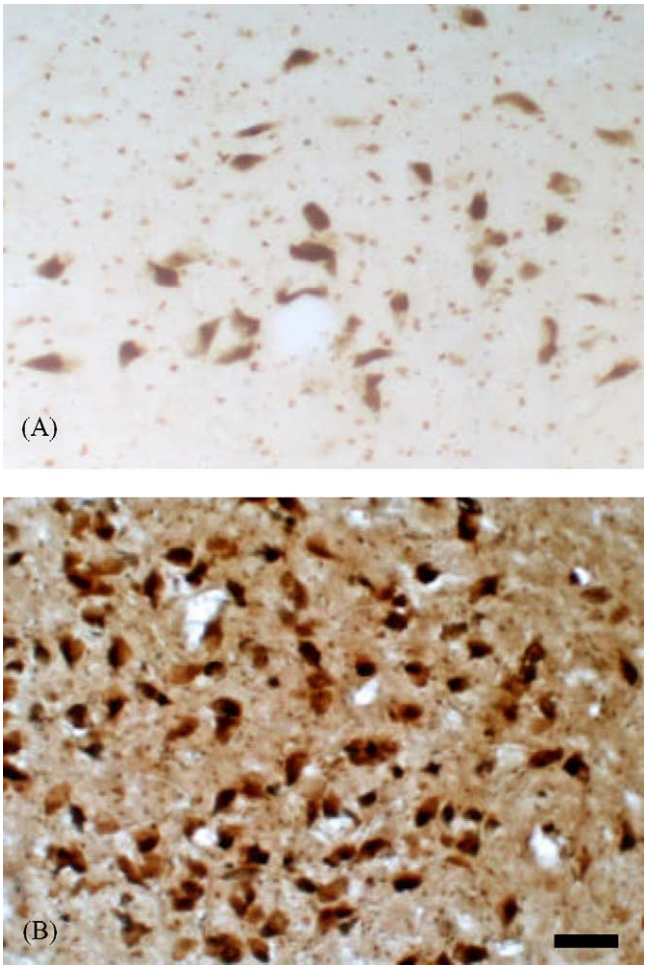

Fig. 6. (A) About one-half the macrocellular neurons within the substantia nigra stain intensely for VDR. (B) A greater percentage of these neurons stain strongly for 1α-OHase. Bar = 50 μm.

4. Discussion

The Vitamin D receptor and the enzyme (1α-OHase) required for the production of the active form of Vitamin D, $1,25(OH)_2D_3$, are found in the adult human brain. Both the receptor and the enzyme were identified in neuronal and glial cells. Most, but not all regions that were positive for 1α-OHase, were also positive for the VDR. A discrete distribution within layers and sub-regions frequently existed for both the receptor and the enzyme. Previous investigators have described a detailed pattern of VDR expression within the brains of experimental animals (Stumpf et al., 1982; Stumpf and O'Brien, 1987; Musiol et al., 1992; Veenstra et al., 1998b; Prufer et al., 1999). The pattern of the distribution of the VDR in the human brain is strikingly similar to that of the rat. VDR expression in human cerebellum is restricted to the granule cells and is completely absent from the Purkinje cells as reported in the rat (Clemens et al., 1988). VDR distribution within the human hippocampus was strongly expressed within CA 1 and 2 pyramidal cells with a marked reduction within CA 3, a finding previously reported in rats by two separate groups (Clemens et al., 1988; Walbert et al., 2001). The concentration of VDR within the supraoptic nucleus and paraventricular nucleus over other nuclei within the hypothalamus has also been reported in rat (Prufer and Jirikowski, 1997). The complete absence of VDR staining in the macrocellular neurons from the nucleus basalis again is confirmed in the rat (Stumpf and O'Brien, 1987) however it must be noted that VDR is present in the same region of

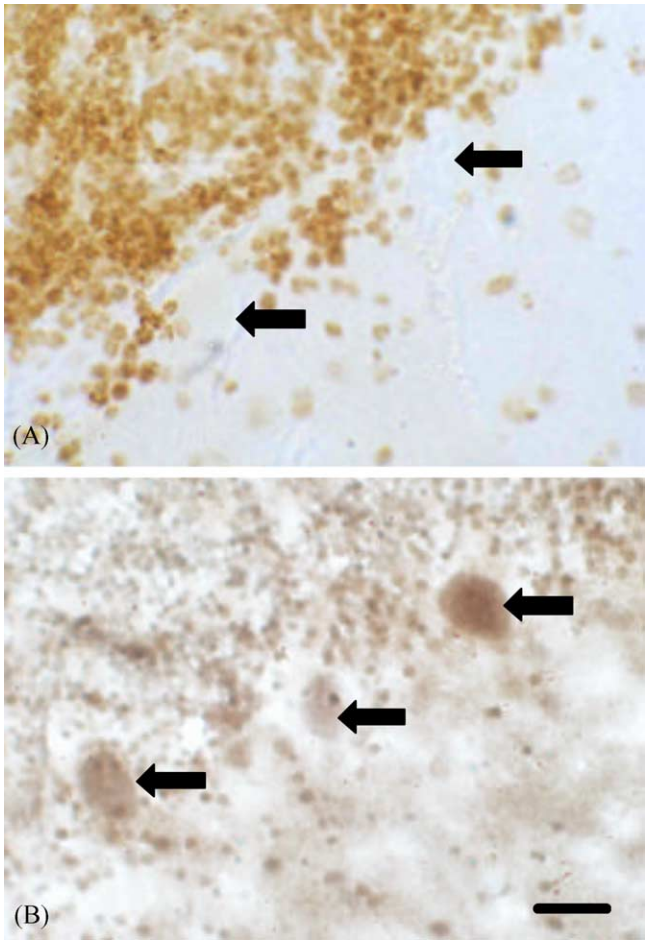

Fig. 7. (A) Negligible VDR staining is seen in Purkinje cells or within the molecular layer whilst prominent staining is seen in the granule cell layer. (B) Strong 1α-OHase immunoreactivity is seen within Purkinje cells (arrows) as well as most cells within the granule and to a lesser extent molecular layers of the cerebellum. Bar = 50 μm.

hamster brains (Musiol et al., 1992). In general the level of expression of VDR in the remaining cortical and non-cortical structures was equivalent with that reported in previous rodent studies (Stumpf and O'Brien, 1987; Prufer et al., 1999).

Two exceptions to this reasonably tight overlap between VDR distribution across species are the apparent lamination of both VDR and 1α-OHase within the external granule layer of the prefrontal cortex and the absence of VDR staining within human amygdala which stands in stark contrast to the consistent presence observed at least in the central nucleus of rats and hamsters (Stumpf et al., 1982; Prufer et al., 1999; Walbert et al., 2001; Musiol et al., 1992). Additionally although in general the immunohistochemical profile of VDR distribution in human brain replicates the distribution in the rat there may still be selective differences in binding affinities amongst these various brain nuclei.

This report has clarified two important issues regarding the distribution of the VDR in brain. Firstly we report in human brain a strict localisation of the immunohistochemical response for VDR within the nucleus of the cell. This is in accordance with the known distribution of this receptor in cells within other organs. There is limited evidence in the literature to support the presence of a cytosolic VDR in the brain. This is provided by mRNA and immunohistochemistry studies in human and rat brain cell cytosol respectively (Sutherland et al., 1992; Prufer et al., 1999). The existence of cytosolic VDR mRNA may simply reflect the presence of message prior to receptor synthesis (Barsony et al., 1997). The cytosolic immunohistochemical reaction noted in the aforementioned rodent study possibly reflects the choice of antibody with those authors reporting a strict nuclear reaction when the same monoclonal antibody used in this study was employed (Prufer et al., 1999). The use of a polyclonal VDR antibody by these authors and in experiments in this laboratory (unpublished), have revealed a more widespread reaction across the cell soma. Whether this reflects an actual de novo cytosolic receptor, newly synthesised protein being transported to the nucleus or simply the reduced specificity of a polyclonal antibody is unknown. Secondly we have clarified that the VDR is present in both neurons and glia. This has generally been assumed to be the case but until now has not been demonstrated by dual labeling studies.

In keeping with two previous studies (Fu et al., 1997; Zehnder et al., 2001), we confirm that 1α-OHase is expressed in the human brain. Both neuronal and glial cells were shown to express this enzyme consistent with the knowledge that 1α-OHase has been shown previously to be both present within cultured human glioma cell lines (Neveu et al., 1994b) and in neurons within human brain (Zehnder et al., 2001). As with the VDR, the pattern of expression of 1α-OHase has regional and sub-regional specificity. The regions that stained the strongest were the supraoptic and paraventricular nuclei within the hypothalamus and the substantia nigra. The rich presence of both the VDR and 1α-OHase in the hypothalamus is consistent with the distribution of other neurosteroids (Prufer and Jirikowski, 1997). The VDR belongs to the phylogenetically conserved family of nuclear receptors, which also include receptors for cortisol, sex hormones, retinoic acid, and thyroid hormone. As there is now strong evidence showing that some steroid hormones have autocrine/paracrine properties in the human brain (Melcangi and Panzica, 2001), it raises the intriguing possibility that Vitamin D may also have "neurosteroid" properties (McGrath et al., 2001).

The strong immunochemical presence of both the VDR and 1α-OHase within the substantia nigra is of particular interest. Vitamin D has been shown to regulate tyrosine hydroxylase gene expression in the adrenyl medulla (Puchacz et al., 1996). Other studies have show that dopamine is able to induce VDR-mediated signaling in the absence of the ligand suggesting a complex interaction between Vitamin D and neurotransmitters (Matkovits and Christakos, 1995). Thus links between Vitamin D and

Table 1
A summary of the immunoreactivity observed within the brain regions examined for both 1α-OHase and VDR

Brain region	VDR immunoreactivity	1α-OHase immunoreactivity
Prefrontal cortex		
Molecular layer	+	+
External granule	+ + +	+ + + +
External pyramidal	+ +	+ +
Internal granule	+ +	+ + +
Internal pyramidal	+ +	+ +
Multiform	+ +	+ +
Cingulate gyrus		
Molecular layer	+ + +	+ +
External granule	+ + +	+ + +
External pyramidal	+ +	+ + +
Internal granule	+ + +	+ + +
Internal pyramidal	+ +	+ + +
Multiform	+ +	+ + +
Hippocampus		
CA1 region		
Molecular layer	+ +	+ +
Pyramidal layer	+ + +	+ + +
Polymorphic layer	+ +	+ +
CA2 region		
Molecular layer	+ +	+
Pyramidal layer	+ + +	+ + +
Polymorphic layer	+ +	+ +
CA3 region		
Molecular layer	+	+
Pyramidal layer	+	+ + +
Polymorphic layer	+	+ +
Fimbria	+ + +	+ + +
Dentate gyrus		
Granule Cell layer	+ + +	+ + +
Molecular layer	+ +	+ +
Polymorphic layer	+ +	+ +
Basal forebrain	−	+ + +
Caudate/putamen	+ + +	+ + +
Amygdala	+	+ +
Thalamus		
Lateral-dorsal region	+ +	+ +
Medial-dorsal region	+ +	+ +
Ventrolateral region	+ +	+ +
Ventromedial region	+ +	+ +
Substantia nigra	+ + + +	+ + + +
Lateral geniculate nuclei	+ + +	+ + +
Hypothalamus		
Supraoptic nucleus	+ + +	+ + + +
Paraventricular nucleus	+ + +	+ + + +
Dorsal region	+ +	+ +
Lateral region	+ +	+ +
Ventromedial region	+ + +	+ +
Cerebellum		
Molecular layer	−	+ +
Purkinje layer	−	+ +
Granule cell layer	+ + +	+ +

Key: (−) none; (+) weak; (+ +) moderate; (+ + +) intense; (+ + + +) very intense immunoreactivity.

dopamine-mediated disorders of movement may warrant further scrutiny.

Paradoxically, two different classes of large neurons expressed 1α-OHase but not the VDR. The first of these, the macrocellular CH4 neurons in basal forebrain provide a large portion of the cholinergic innervation of the cortex. They are known to be rich with a protein strongly regulated by Vitamin D in non-neuronal tissues (calbindin 28K Vitamin D-dependent protein). In addition, these cells rely on retrograde transport of nerve growth factor, a neurotrophin whose expression is known to be tightly linked to Vitamin D levels (Wion et al., 1991; Musiol and Feldman, 1997). The other large neurons that were positive for 1α-OHase but not VDR are the Purkinje cells of the cerebellum which are also rich in calbindin. The expression of 1α-OHase within these two types of cells despite the apparent absence of VDR within these cells suggests a paracrine rather than autocrine mode of action. Similar mechanisms have been described for 1α-OHase in macrophages (Kreutz et al., 1993) and dendritic cells (Hewison et al., 2003). In both of these cell types 1α-OHase was up-regulated during differentiation whereas VDR levels declined, suggesting that the cells that are actively producing $1,25(OH)_2D_3$ are not necessarily the ones that respond to it. At least in the case of dendritic cells, this may act as part of a feedback control mechanism to limit an overactive immune response as $1,25(OH)_2D_3$ actively suppresses further dendritic cell development provided the cells express VDR (Griffin et al., 2001; Hewison et al., 2003, 2004). Whether some similar mechanism is acting in CH4 neurons or Purkinje cells remains unclear.

There is now convincing evidence that Vitamin D is important in brain development (Brown et al., 2003; Burkert et al., 2003; Eyles et al., 2003; McGrath et al., 2003; Mackay-Sim et al., 2004). The widespread but distinctive distribution of the receptor for this ligand and the final catabolic enzyme in its synthesis in the human brain raises many questions regarding the function of this steroid in the adult brain. Recently, a number of possible roles for Vitamin D within the brain have been proposed, including the potential of Vitamin D to act in a similar way to neuroactive steroids by modulating neuronal excitability (Zakon, 1998; Rupprecht and Holsboer, 1999). Furthermore, several investigators have shown that Vitamin D can regulate the production of specific neurotrophins. Nerve growth factor synthesis can be regulated by Vitamin D (Neveu et al., 1994b; Saporito et al., 1994; Cornet et al., 1998; Brown et al., 2003) as can neurotrophin 3 (Neveu et al., 1994a) and glial cell-line derived neurotrophic factor (Naveilhan et al., 1996). Several investigators have reported neuroprotective effects of Vitamin D in rodent models of excitotoxicity (Brewer et al., 2001), encephalomyelitis (Garcion et al., 1997), inflammation (Garcion et al., 1998) and exposure to neurotoxins (Shinpo et al., 2000; Wang et al., 2001). The processes underpinning Vitamin D mediated neuroprotection are not fully understood, however mechanisms involving stimulation of neurotrophin production (Riaz et al., 1999; Wang et al., 2000), inhibition of inducible nitric oxide synthase production (Garcion et al., 1997, 1998) or the induction of calcium binding protein production (de Viragh et al., 1989) have all been proposed.

In view of the presence of both the VDR and 1α-OHase in the human brain, and of the evidence that Vitamin D is involved in unexpectedly diverse roles in the brain, this area warrants closer scrutiny in research related to the causes and treatment of neuropsychiatric disorders.

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