Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Steroid Biochemistry & Molecular Biology 89–90 (2004) 557–560
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

Vitamin D_3—implications for brain development☆

John J. McGrath[a,b,*], François P. Féron[a,c], Thomas H.J. Burne[a,c], Alan Mackay-Sim[c], Darryl W. Eyles[a,d]

[a] *Queensland Centre for Schizophrenia Research, The Park Centre for Mental Health, Wacol, Qld 4076, Australia*
[b] *Department of Psychiatry, University of Queensland, Qld 4072, Australia*
[c] *Centre for Molecular Neurobiology, School of Biomolecular and Biomedical Science, Griffith University, Qld 4111, Australia*
[d] *Department of Physiology and Pharmacology, University of Queensland, Qld 4072, Australia*

Abstract

There is growing evidence that 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) is active in the brain but until recently there was a lack of evidence about its role during brain development. Guided by certain features of the epidemiology of schizophrenia, our group has explored the role of $1,25(OH)_2D_3$ in brain development using whole animal models and in vitro culture studies. The expression of the vitamin D receptor (VDR) in the embryonic rat brain rises steadily between embryonic day 15–23, and $1,25(OH)_2D_3$ induces the expression of nerve growth factor and stimulates neurite outgrowth in embryonic hippocampal explant cultures. In the neonatal rat, low prenatal vitamin D_3 in utero leads to increased brain size, altered brain shape, enlarged ventricles, reduced expression of nerve growth factors, reduced expression of the low affinity p75 receptor and increased cellular proliferation. In summary, there is growing evidence that low prenatal levels of $1,25(OH)_2D_3$ can influence critical components of orderly brain development. It remains to be seen if these processes are of clinical relevance in humans, but in light of the high rates of hypovitaminosis D in pregnant women and neonates, this area warrants further scrutiny.

Keywords: 1,25-Dihydroxyvitamin D_3; Vitamin D receptor; Brain growth and development; Prenatal exposures; Nerve growth factor

1. Introduction

Over recent decades there has been an ever-widening range of physiological actions associated with 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). Apart from the classical properties related to calcium regulation and bone growth, research has demonstrated that $1,25(OH)_2D_3$ plays an important role in proliferation and differentiation in many tissues [1]. Of particular interest is the accumulating evidence demonstrating a role for $1,25(OH)_2D_3$ in brain functioning [2]. Our group has a particular interest in the links between prenatal and early life hypovitaminosis D and brain development. The aim of this review is to provide a brief overview of our findings and outline future research directions. The first section will summarise how clues from schizophrenia epidemiology led us to examine the role of $1,25(OH)_2D_3$ in brain development. The middle section of the paper will discuss how our in vitro studies have built on existing knowledge about the role of $1,25(OH)_2D_3$ in the brain. The final section will outline the results of our recent whole animal studies.

2. Clues from the epidemiology of schizophrenia

Schizophrenia is a group of imperfectly understood brain disorders characterised by alterations in higher functions related to perception, cognition communication, planning and motivation. The disorder is characterised by hallucinations, delusions, thought disorder and negative symptoms such as blunted affect and reduced speech [3]. The lifetime morbid risk of schizophrenia is approximately 1 in a 100. The symptoms of the disorder usually emerge in early adulthood and while many individuals with this disorder make a good recovery, many have persistent symptoms and ongoing disability.

There is substantial evidence showing that schizophrenia is associated with abnormal early brain development. The "neurodevelopmental hypothesis" suggests that there is an interaction between genetic and environmental factors during critical periods of development that affect later brain function [4]. The clinical sequelae related to this

☆ Presented at the 12th Workshop on Vitamin D (Maastricht, The Netherlands, 6–10 July, 2003).

* Corresponding author. Tel.: +61-7-3271-8694; fax: +61-7-3271-8698.
E-mail address: john_mcgrath@qcsr.uq.edu.au (J.J. McGrath).

0960-0760/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2004.03.070

altered brain development are not obvious until after puberty, when brain maturation leads to the emergence of symptoms.

Recently, we proposed that low prenatal $1,25(OH)_2D_3$ may be a risk-modifying factor for schizophrenia [5]. This hypothesis can parsimoniously explain three key epidemiological features of schizophrenia. Firstly, people born in winter and spring have a small but significantly higher chance of developing schizophrenia [6]. Levels of 25-dihydroxyvitamin D_3 are lower in winter and early spring. Secondly, studies from the United Kingdom and the Netherlands have found an increased incidence of schizophrenia in the offspring of dark-skinned migrants [7,8]. This group is particularly vulnerable to hypovitaminosis D at high latitudes due to the darker skin pigmentation. Finally, several studies have shown that those born in the city have an increased risk of schizophrenia compared to those born in rural settings [9,10]. City dwellers tend to have less exposure to ultraviolet radiation, and thus have lower 25-dihydroxyvitamin D_3 levels [11].

3. Vitamin D_3 and the brain

While clues from epidemiology suggest that low prenatal $1,25(OH)_2D_3$ might be a candidate risk-modifying factor, is it a biologically plausible candidate? In other words, what is the biological evidence linking $1,25(OH)_2D_3$ and brain development?.

The vitamin D receptor (VDR) has been identified in the brains of the rat [12,13] and hamster [14]. VDR is expressed widely in the adult brain in temporal, orbital and cingulate cortices, in the thalamus, in the accumbens nuclei, parts of the stria terminalis and amygdala and widely throughout the olfactory system. It was also expressed in pyramidal neurons of the hippocampal regions CA1, CA2, CA3, CA4 [13]. Curiously, there is scant evidence showing that the VDR is expressed in the human brain. To the best of our knowledge, only one study has actually examined the VDR in human post-mortem brain tissue [15]. Despite this lack of evidence, we expect that the VDR will be located in the human brain in a distribution comparable to other mammals.

The VDR is widely distributed throughout the embryonic rat brain, most prominently in the neuroepithelium and proliferating zones of the rat CNS on embryonic days 12, 15, 18 and 21 respectively [16]. In particular, this paper comments on the association between the presence of the VDR in differentiating zones and mitotic activity in these regions.

Using quantitative methods we recently examined the expression of VDR mRNA and protein during rat brain development (embryonic days 15–23) [17]. We found that VDR expression dramatically increases at embryonic day 18, which correlates with the well-described increase in apoptosis and decrease in mitosis at this time. We cannot prove a causal association between the appearance of the VDR and these changes, but we speculate that the VDR may help regulate apoptosis and mitosis at this time.

Apart from the presence of the receptor, enzymes involved in the hydroxylation of vitamin D (vitamin D_3 25-hydroxylase and 25-hydroxyvitamin D_3-1-hydroxylase) and the inactivating enzyme (vitamin D_3 24-hydroxylase) are also present in the brain, suggesting that $1,25(OH)_2D_3$ may have autocrine or paracrine properties in this organ [2,18], and may meet the criteria for a neurosteriod [19]. Garcion and colleagues have recently published a scholarly review of the role of $1,25(OH)_2D_3$ in the brain [2] which concludes that this molecule has a wide range of effects on adult brain tissue, in vivo and in vitro. For example $1,25(OH)_2D_3$ is a potent promoter of GDNF [20], NT3 [21] and NGF [22–25]. Vitamin D_3 responsive elements are also present in the promoter region of the low affinity neurotrophin receptor $p75^{NTR}$ gene [20].

Recently, our group has shown that in vitro, $1,25(OH)_2D_3$ regulates mitosis and neurite outgrowth in embryonic day 18 hippocampal explant cultures [26]. $1,25(OH)_2D_3$ significantly decreased cell proliferation in these cultures, consistent with its action on non-CNS cells [27]. Additionally, $1,25(OH)_2D_3$ increased neurite outgrowth and increased the expression of NGF in these cultures.

In summary, there is abundant circumstantial evidence to suggest that $1,25(OH)_2D_3$ influences brain development, but until recently, there was no direct evidence of this link. In the next section of this review, we will outline the results of our recent studies that demonstrate this link more directly.

4. Hypovitaminosis D affects brain development

Over the last 3 years we have examined the impact of $1,25(OH)_2D_3$ deficiency during embryonic and early life on brain development in the rat [28]. Pups were born to mothers deprived of $1,25(OH)_2D_3$. At birth, mothers were fed diets containing $1,25(OH)_2D_3$. The pups were assessed at birth and at 10 weeks of age. Another group of pups were deprived of $1,25(OH)_2D_3$ until weaning and then placed on the normal $1,25(OH)_2D_3$ -containing diet. The control group were born and weaned to mothers fed the normal $1,25(OH)_2D_3$-containing diet. We examined gross brain morphology, cell division and cell death, the expression of various neurotrophins and their receptors, and behaviour. In this review paper we summarise the results of the first wave of experiments that demonstrate the impact of low prenatal $1,25(OH)_2D_3$ on the neonatal rat brain.

Curiously, vitamin D_3-depleted pups were heavier than the control animals although the ratio of brain to body weight did not differ between the groups. Vitamin D_3-depleted pups had cerebral hemispheres that were longer but not wider than controls, leading to a larger length/width ratio. These pups had lateral ventricles that were double that of the controls even when corrected for the increased hemispheric volume. Finally, the neocortex of these pups was thinner

than controls after the data were normalised for whole-brain cross-sectional area.

There was more cell proliferation in the brains of the vitamin D_3-depleted pups compared to controls, without differences in cell density or apoptosis at birth. These findings are consistent with the known pro-differentiating and pro-apoptotic properties of $1,25(OH)_2D_3$ [27]. In the absence of $1,25(OH)_2D_3$, the proliferation of neurons continues unchecked, resulting in larger brains with an altered shape.

As we predicted from our in vitro studies, vitamin D_3 depletion in utero led to significantly reduced levels of NGF at birth (a 17% reduction compared to controls). None of the other three neurotrophins (BDNF, NT-3 and NT-4) were affected by vitamin D_3 depletion, nor were the neurotrophin tyrosine kinase receptors, although we observed a marked decrease in expression of $p75^{NTR}$, the low-affinity neurotrophin receptor. It is evident that $1,25(OH)_2D_3$ acts on the developing brain via the neurotrophin signalling pathway, but vitamin D_3 depletion also reduced glial cell line-derived neurotrophic factor (GDNF) expression by 25% compared to controls [28]. $1,25(OH)_2D_3$ is also reported to interact with the transforming growth factor β family signalling pathway [29], and the nonreceptor protein kinase pathway [30]. Thus there is scope for $1,25(OH)_2D_3$ to act on brain development in a cell- and tissue-specific manner, depending not only on the expression of VDR-target genes but also with interactions on several growth factor signalling pathways.

5. Conclusions and implications for future research

In summary, these findings indicate that prenatal vitamin D_3 depletion can lead to changes in many features of brain development (morphology, cellular proliferation, neurotrophin systems). Currently our group is examining the impact of prenatal vitamin D_3 depletion in the adult rat and extending the studies by examining adult behaviours. In addition, we are exploring aspects of brain development and behaviour in the VDR knock-out mouse. It remains to be proven if the effects of prenatal Vitamin D_3 depletion on brain development persist into adulthood after $1,25(OH)_2D_3$ levels are normalised, but the effects observed provide a plausible biological mechanism of action for adult-onset behavioural disorders. Nevertheless, apart from clues from epidemiology [31], we do not have strong evidence that low prenatal $1,25(OH)_2D_3$ impacts on the human brain, nor that it is related to any adverse neuropsychiatric outcomes. We are currently exploring this issue using more analytic epidemiological research designs (e.g. case-control studies comparing 25OH D_3 in banked maternal sera or in neonatal dried whole blood spots).

Although we are specifically examining the impact of low prenatal and early life hypovitaminosis D on adult neuropsychiatric outcomes, early life hypovitaminosis D may play a greater role than previously suspected in a range of other disorders, as suggested by epidemiological evidence in multiple sclerosis, type I diabetes, breast cancer, prostate cancer, colorectal cancer, and osteoporosis [32]. In other words, we speculate that low $1,25(OH)_2D_3$ during early life may also play a role in modifying the risk of a range of adult-onset disorders. If this is the case, then the potential importance of an association between early life $1,25(OH)_2D_3$ levels and organ development takes on added weight in view of the surprisingly high levels of hypovitaminosis D in both developed and developing nations [32,33]. Pregnant women and their offspring are prone to hypovitaminosis D because of the reduced outdoor activity during pregnancy and lactation and because of the increased physiological needs during pregnancy. In the United States it was reported that, of women of the child-bearing ages 20–39, 12% had serum 25-hydroxyvitamin D_3 levels below the threshold defined for vitamin D_3 deficiency (15 ng/ml) [34]. If low prenatal vitamin D is linked to adverse neuropsychiatric outcomes, then the hypothesis has important public health implications. While highly speculative, it is possible that optimizing $1,25(OH)_2D_3$ levels in pregnant women could lead to a reduced incidence of schizophrenia, analogous to the reduced incidence of neural tube defects by folate supplementation.

Acknowledgements

The Stanley Medical Research Institute and the National Health and Medical Research Council supported this project.

References

[1] R. Bouillon, W.H. Okamura, A.W. Norman, Structure-function relationships in the vitamin D endocrine system, Endocr. Rev. 16 (1995) 200–257.
[2] E. Garcion, N. Wion-Barbot, C.N. Montero-Menei, F. Berger, D. Wion, New clues about vitamin D functions in the nervous system, Trends. Endocrinol. Metabol. 13 (2002) 100–105.
[3] American Psychiatric Association, DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, fourth ed., Washington, D.C., The Association, 1994.
[4] J.J. McGrath, F.P. Feron, T.H. Burne, A. Mackay-Sim, D.W. Eyles, The neurodevelopmental hypothesis of schizophrenia: a review of recent developments, Ann. Med. 35 (2003) 86–93.
[5] J. McGrath, Hypothesis: is low prenatal vitamin D a risk-modifying factor for schizophrenia? Schizophr. Res. 40 (1999) 173–177.
[6] G. Davies, J. Welham, D.C. Chant, E.F. Torrey, J. McGrath, Season of birth effect and latitude: a systematic review and meta-analysis of Northern Hemisphere studies, Schizophr Bull. 29 (2003) 587–593.
[7] G. Harrison, Searching for the causes of schizophrenia: the role of migrant studies, Schizophr. Bull. 16 (1990) 663–671.
[8] J.P. Selten, J. Slaets, R. Kahn, Prenatal exposure to influenza and schizophrenia in Surinamese and Dutch Antillean immigrants to The Netherlands, Schizophr. Res. 30 (1998) 101–103.
[9] M. Marcelis, F. Navarro-Mateu, R. Murray, J.-P. Selten, J. van Os, Urbanization and psychosis: a study of 1942–1978 birth cohorts in The Netherlands, Psychol. Med. 28 (1998) 871–879.

[10] P.B. Mortensen, C.B. Pedersen, T. Westergaard, J. Wohlfahrt, H. Ewald, O. Mors, P.K. Andersen, M. Melbye, Effects of family history and place and season of birth on the risk of schizophrenia, N. Eng. J. Med. 340 (1999) 603–608.
[11] J.J. McGrath, M.G. Kimlin, S. Saha, D.W. Eyles, A.V. Parisi, Vitamin D insufficiency in south-east Queensland, Med. J. Aust. 174 (2001) 150–151.
[12] W.E. Stumpf, L.P. O'Brien, 1,25 $(OH)_2$ vitamin D3 sites of action in the brain. An autoradiographic study, Histochemistry 87 (1987) 393–406.
[13] K. Prüfer, T.D. Veenstra, G.F. Jirikowski, R. Kumar, Distribution of 1,25-dihydroxyvitamin D3 receptor immunoreactivity in the rat brain and spinal cord, J. Chem. Neuroanat. 16 (1999) 135–145.
[14] I.M. Musiol, W.E. Stumpf, H.J. Bidmon, C. Heiss, A. Mayerhofer, A. Bartke, Vitamin D nuclear binding to neurons of the septal, substriatal and amygdaloid area in the Siberian hamster (Phodopus sungorus) brain, Neuroscience 48 (1992) 841–848.
[15] M.K. Sutherland, M.J. Somerville, L.K. Yoong, C. Bergeron, M.R. Haussler, D.R. McLachlan, Reduction of vitamin D hormone receptor mRNA levels in Alzheimer as compared to Huntington hippocampus: correlation with calbindin-28k mRNA levels, Brain Res. Mol. Brain Res. 13 (1992) 239–250.
[16] T.D. Veenstra, K. Prüfer, C. Koenigsberger, S.W. Brimijoin, J.P. Grande, R. Kumar, 1,25-Dihydroxyvitamin D3 receptors in the central nervous system of the rat embryo, Brain Res. 804 (1998) 193–205.
[17] R. Burkert, J. McGrath, D.W. Eyles, Vitamin D receptor expression in the embryonic rat brain, Neurosci. Res. Comm. 33 (2003) 67–71.
[18] W.L. Miller, A.A. Portale, Vitamin D 1alpha-Hydroxylase, Trends Endocrinol. Metabol. 11 (2000) 315–319.
[19] J. McGrath, F. Feron, D. Eyles, Vitamin D: the neglected neurosteroid? Trends Neurosci. 24 (2001) 570–572.
[20] P. Naveilhan, I. Neveu, C. Baudet, H. Funakoshi, D. Wion, P. Brachet, M. Metsis, 1,25-Dihydroxyvitamin D3 regulates the expression of the low-affinity neurotrophin receptor, Brain Res. Mol. Brain Res. 41 (1996) 259–268.
[21] I. Neveu, P. Naveilhan, C. Baudet, P. Brachet, M. Metsis, astrocytes, 1,25-dihydroxyvitamin D3 regulates NT-3, NT-4 but not BDNF mRNA in Neuroreport 6 (1994) 124–126.
[22] I. Neveu, P. Naveilhan, F. Jehan, C. Baudet, D. Wion, H.F. De Luca, P. Brachet, 1,25-dihydroxyvitamin D3 regulates the synthesis of nerve growth factor in primary cultures of glial cells, Brain Res. Mol. Brain Res. 24 (1994) 70–76.
[23] M.S. Saporito, H.M. Wilcox, K.C. Hartpence, M.E. Lewis, J.L. Vaught, S. Carswell, Pharmacological induction of nerve growth factor mRNA in adult rat brain, Exp. Neurol. 123 (1993) 295–302.
[24] D. Wion, D. MacGrogan, I. Neveu, F. Jehan, R. Houlgatte, P. Brachet, 1,25-Dihydroxyvitamin D3 is a potent inducer of nerve growth factor synthesis, J. Neurosci. Res. 28 (1991) 110–114.
[25] I.M. Musiol, D. Feldman, 1,25-dihydroxyvitamin D3 induction of nerve growth factor in L929 mouse fibroblasts: effect of vitamin D receptor regulation and potency of vitamin D3 analogs, Endocrinology 138 (1997) 12–18.
[26] J. Brown, J.I. Bianco, J.J. McGrath, D.W. Eyles, 1,25-Dihydroxyvitamin D3 induces nerve growth factor, promotes neurite outgrowth and inhibits mitosis in embryonic rat hippocampal neurons, Neurosci. Lett. 343 (2003) 139–143.
[27] H.F. DeLuca, J. Krisinger, H. Darwish, The vitamin D system, Kidney Int. Suppl. 29 (1990) S2–S8.
[28] D. Eyles, J. Brown, A. Mackay-Sim, J. McGrath, F. Feron, Vitamin D3 and brain development, Neuroscience 118 (2003) 641–653.
[29] J. Yanagisawa, Y. Yanagi, Y. Masuhiro, M. Suzawa, M. Watanabe, K. Kashiwagi, T. Toriyabe, M. Kawabata, K. Miyazono, S. Kato, Convergence of transforming growth factor-beta and vitamin D signalling pathways on SMAD transcriptional coactivators, Science 283 (1999) 1317–1321.
[30] R. Gniadecki, Nongenomic signalling by vitamin D: a new face of Src, Biochem. Pharmacol. 56 (1998) 1273–1277.
[31] J. McGrath, D. Eyles, B. Mowry, R. Yolken, S.L. Buka, Low maternal vitamin D as a risk factor for schizophrenia: a pilot study using banked sera, Schizophr. Res. 63 (2003) 73–78.
[32] J. McGrath, Does 'imprinting' with low prenatal vitamin D contribute to the risk of various adult disorders, Med. Hypotheses 56 (2001) 367–371.
[33] R. Vieth, G. Carter, Difficulties with vitamin D nutrition research: objective targets of adequacy, and assays for 25-hydroxyvitamin D, Eur. J. Clin. Nutr. 55 (2001) 221–222.
[34] A.C. Looker, E.W. Gunter, Hypovitaminosis D in medical inpatients, N. Engl. J. Med. 339 (1998) 344–345.