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Aberrant nest building and prolactin secretion in vitamin D receptor mutant mice☆

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Abstract

1α,25(OH)$_2$D$_3$, the hormonal form of vitamin D, is a neuroactive seco-steroid hormone with multiple functions in the brain. Most of these effects are mediated through the nuclear vitamin D receptor (VDR), widely distributed in the central nervous system. Our earlier studies showed that mutant mice lacking functional VDR have specific behavioural abnormalities, including anxiety and aberrant maternal behaviour, which may be hormonally regulated. Here we describe impaired nest building behaviour in VDR mutant mice. Since prolactin plays a key role in the regulation of nest building in both sexes, we also examine whether VDR mutant mice have altered prolactin levels. Overall, serum prolactin levels were increased in VDR mutant mice, accompanied by marked impairments in their nest building activity. In contrast, there were no differences in prolactin mRNA expression levels between wildtype control mice and VDR mutant mice. Collectively, these data suggest that partial genetic ablation of VDR affects prolactin system in mice, and that altered serum prolactin levels in VDR mutants may underlie some of their behavioural abnormalities, such as impaired nest building.

Keywords: Vitamin D receptor; Prolactin; Nest building behaviour; Mutant mice

1. Introduction

1α,25(OH)$_2$D$_3$ (calcitriol), is neurosteroid hormone that plays an important role in the nervous system [1–3]. The effects of calcitriol are mediated through its interaction with a high-affinity nuclear vitamin D receptor (VDR), a member of the nuclear receptors super-family of ligand-activated transcription factors [4–6]. VDRs are widespread in the brain and the spinal cord including the areas involved in the regulation of motor activity and behaviour [7–10]. Collectively, this implies a possible role of calcitriol and VDR in the regulation of behaviour [11–13,2,14–16].

Mice lacking functional VDR emerge as a useful tool to study physiological role of vitamin D [17,18]. These mice display high anxiety, abnormal grooming and impaired social behaviours [19,14–16]. Although earlier studies in mice lacking functional VDR revealed impaired fertility [20], these mice are fertile when kept on a special Ca/P rich diet [21], but display impaired maternal (pup-eating, poorer mothering style) and nest building behaviours [19].

In addition to "maternal" nest building in females, both mouse sexes build nests to be used as a shelter [22] or for thermoregulation [23]. Our earlier study also showed that non-maternal nest building was impaired in VDR mutant mice of both sexes, although males of both genotypes showed higher nest building activity than did their female littermates [19]. Since prolactin is implicated in nest building behaviour [24,25], and the vitamin D system interacts with prolactin [26,27], we hypothesized that prolactin neuroendocrine system may be affected in VDR mutant mice [19].

As prolactin is highly regulated by estrogen in female mice [24], while male mice showed higher nest building activity, males may represent a better model to study this behaviour in our genetic model. Here we assess nest building behavior in

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VDR mutant male mice, and examine whether they differ in serum prolactin or in prolactin mRNA (PRL mRNA) expression levels, compared to wild type male mice.

2. Materials and methods

2.1. Animals

The management of mice and experimental procedures in this study were approved by the Ethical Committee of the University of Tampere and performed according to EU legislation. Subjects were male mice (35–47 weeks old) on 129S1 background strain. Mice were kept in a virus/parasite-free facility under controlled temperature (22 ± 2 °C) and humidity (60%) with 12 h light, 12 h dark cycle. The animals were housed individually in transparent cages (13 cm × 12 cm × 14 cm) with food and water available *ad libitum*. VDR mutant mice were bred from the line originally generated in the University of Tokyo (Tokyo, Japan) [18] and fed with special rescue Ca/P-rich diet (Lactamin AB, Kimstad, Sweden) to normalize mineral homeostasis.

Genotyping of the mice was confirmed by PCR on DNA prepared from tail tissue. Four primers were used to amplify a 166 bp VDR (forward, 5′-CTG CTC TTC TTA CAG GGA TGG-3′and reverse, 5′-GAC TCA CCT GAA GAA ACC CTT G-3′) and 400 bp Neo (forward, 5′-ATC TTC TGT CAT CTC ACC TTG C-3′and reverse, 5′-CAA GCT CTT CAG CAA TAT CAC G-3′) band from the targeted allele. After testing, the mice were sacrificed using carbon dioxide. Blood was taken for prolactin measurement by heart puncture, and pituitary gland, kidney and liver tissue samples were taken for PRL mRNA measurements ($n = 10$ in each group).

2.2. Nest building experiment

A standard piece of paper towel (23 cm × 23 cm) was placed in each cage for 4 days, and then assessed by two observers (intra- and inter-rater reliability > 0.9), using scoring system described previously [19]: 0 = no nest, 1 = primitive flat nest (flat paper slightly elevated from the bedding), 2 = more complex nest (wrapping and biting the paper), 3 = complex accurate cup-shaped nest (walls and shredded paper), 4 = complex hooded nest. The amount of paper damage was also assessed here, using the following scale: 0 = intact paper or little damage (<5% paper destroyed), 1 = some paper damage (5–20%), 2 = pronounced paper damage (20–40%), 3 = severe paper damage (>40%) [19].

2.3. Serum prolactin

Serum was separated by centrifugation and stored at −20 °C until hormone measurement. Prolactin levels were determined from serum samples by double-antibody radioimmunoassay (RIA) [28]. The sensitivity of the assay was 0.02 pg/tube, intra-assay coefficients of variance (CV) < 5–8% and inter-assay CV < 15%.

2.4. RNA isolation, primer design, cDNA synthesis and quantitative real-time PCR

Pituitary gland, kidney and liver samples were placed in Trizol®-solution for homogenization. Total RNA was isolated by following the manufacturer's instructions. Total RNA amounts were quantified by measuring the absorbance at 260 nm.

As recommended in the manufacturer's protocol, primers were designed using Primer Express v2.0 software (Perkin-Elmer Applied Biosystems, Foster City, California USA). Mouse PRL mRNA expression in the tissues was detected using real-time PCR. The cDNA was synthesized from the total RNA by reverse transcription PCR using a High Capacity cDNA Archive kit (Applied Biosystems, Foster City, California USA). The reaction was performed at 37 °C for 2 h. Samples were stored at −20 °C prior to the real-time PCR reaction. Target cDNA for mPRL was amplified by PCR for 40 cycles (1 cycle: 95 °C for 15 s, 60 °C for 1 min) in ABI PRISM® 7000 SDS using 20 ng of SYBR® Green solution (Applied Biosystems, Foster City, California USA). The mPRL primers used in this study equate to following mouse mPRL cDNA sequences: 5′-CTCTCAGGCCATCTTGGAGAA-3′ (forward) and 5′GGCTGACCCCTGGCTGTT-3′ (reverse) (10 μM). To estimate the quality of RNA and to calculate the relative expression ratio of mPRL, the β-actin cDNA in each samples were also amplified by PCR using β-actin primers according to the mouse sequence: 5′-GCTTCTTTGCAGCTCCTTCGT-3′ (forward) and 5′-CCAGCGCAGCGATATCG-3′ (reverse) (10 μM).

2.5. Statistics

All results are presented as mean ± S.E.M. Data were analyzed using the Mann–Whitney *U*-test for independent samples. A probability of less than 0.05 was considered statistically significant in all tests.

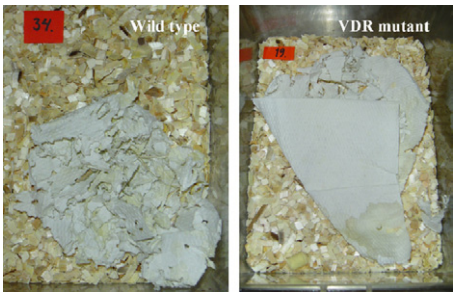

Fig. 1. Representative nests of the wild type and VDR mutant mouse.

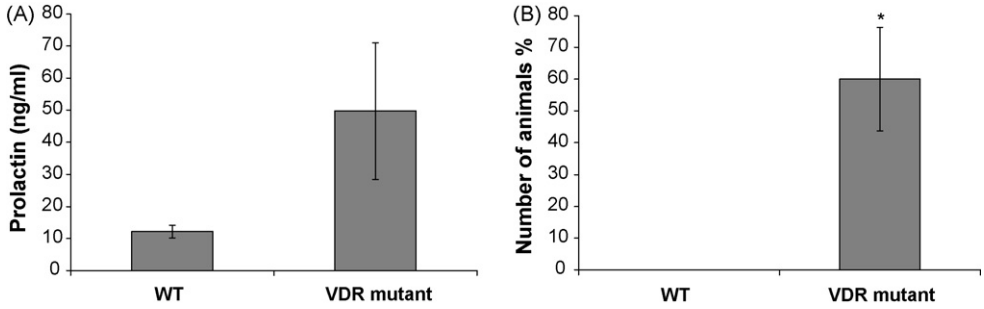

Fig. 2. (A) Serum prolactin concentrations in VDR mutant and wildtype male mice ($n = 10$). Data presented as mean ± S.E.M. (B) Percentage of animals with elevated (>25 ng/ml) levels of prolactin (*$P < 0.05$, *U*-test between groups).

3. Results

3.1. Nest building test

Overall, the wild type male mice produced significantly more paper damage (2.7 ± 0.15; $n = 10$) than did their VDR mutant littermates (1.8 ± 0.20; $n = 10$; $P < 0.005$). In addition, the wild type mice tended to build more complex and accurate nests compared to VDR mutant littermates (2.2 ± 0.13 versus 1.7 ± 0.21, respectively; $n = 10$ in each group) (Fig. 1), although this difference did not reach significance ($P < 0.06$).

3.2. Serum prolactin

Analyzing serum prolactin levels, we found no significant difference between the genotypes, although VDR mutant mice generally displayed a clear tendency to elevated serum prolactin (Fig. 2A). Notably, six VDR mutant mice out of 10 showing markedly higher (>25 ng/ml) prolactin levels (78.7 ± 30.6 ng/ml ($n = 6$) versus 5.8 ± 1.0 ng/ml, ($n = 4$)); $P < 0.01$ between subgroups; $P < 0.05$ for the number of animals with high prolactin levels versus the wild type mice (Fig. 2B). In the wild type group, however, this variation was generally smaller, and there were no samples >25 ng/ml, thus significantly differing from the VDR mutant group ($P < 0.03$).

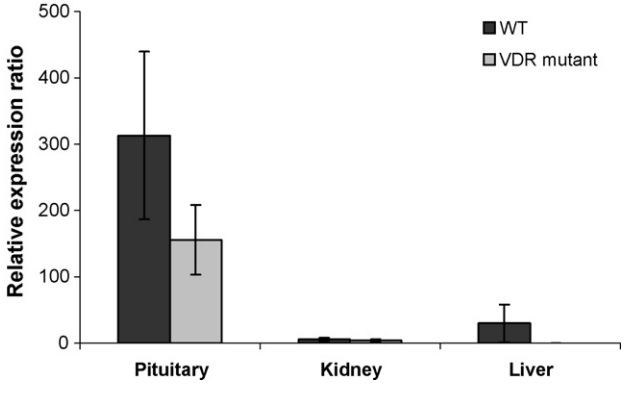

Fig. 3. Relative expression ratios of PRL mRNA in the wildtype (WT) and VDR mutant male mice in pituitary ($n = 9$ and $n = 6$, respectively), kidney ($n = 7$) and liver ($n = 8$ and $n = 5$).

3.3. Relative expression levels of PRL mPRL

Prolactin expression levels in this study were calculated using mathematical model for relative quantification in real-time RT-PCR [29]. The amount of target mRNA was calculated relative to β-actin mRNA, and all measures were compared to one control sample. Prolactin mRNA expression levels varied between tissues (Fig. 3.), but there was no difference in the prolactin expression levels between the genotypes.

4. Discussion

In nest building experiment, we found significant difference in the amount of paper damage between the two genotypes, confirming impaired nest building behavior in VDR mutant mice. Likewise, the VDR mutants also tended to build less complete nests (compared to the wild type mice), which is also in agreement with our earlier findings in these mice [19]. As shown by Bula et al. [30], our VDR mutant mouse strain expresses VDR protein that retains the capability to bind ligands but does not bind to DNA. The results of this study seem to support the notion that the regulation of nest building behaviour seems to require the VDR binding to vitamin D-responsive elements, and thus regulation of gene transcription by VDR.

Overall, one possibility to explain these findings may be that deficits of nest building are associated with motor impairments [31,32], and the latter, sometimes observed in our VDR mutant mice [15], may contribute to their insufficient nest completeness (Fig. 1).

Another likely possibility can be anxiety, previously reported in this genetic mouse model [16]. Since stress is involved in the regulation of prolactin, while its levels are elevated in animals with increased stress or anxiety [33], and reduced by anxiolytic drugs [34,35], elevated prolactin may underlie the VDR mutant mouse anxiety, raising the possibility that their neophobic behavior (in combination with reduced activity) may collectively lead to their poorer nest building performance. Interestingly, in some studies [36] stress increases prolactin secretion without altering PRL

mRNA levels, thus strikingly paralleling our findings. Taken together, these data support the notion that elevated prolactin in VDR mutant mice may be the result of chronic stress or anxiety in these mice, and that this possibility may represent an interesting direction of further research in this field.

The difference between serum prolactin levels in VDR mutant mice reported here (Fig. 2) is an interesting finding, and may be due high pulsation secretion of prolactin from the pituitary. The fact that the variability in the wild type male group was much lower indicates that VDR mutant mice may have abnormally high pulsation amplitude of secretion, suggesting prolactin endocrine dysregulation and the possibility that both VDR- and prolactin-dependent mechanisms may contribute to this phenomenon.

Interestingly, Binart et al. [37] have noted that prolactin may have a direct regulation of its own secretion. High levels of serum prolactin and diminished prolactin behavioral effects on nest building observed here in the VDR mutant mice suggest possible reduction of prolactin receptors (induced by its direct neuroendocrine feedback control [38]). In line with this hypothesis, abnormally high serum prolactin levels have previously been described in prolactin receptor (PRLR) knockout and in dopamine D2 receptor-deficient mice [37,39]. Finally, dopamine is one of the major prolactin-inhibiting factors [40], and it has been suggested that high prolactin levels in PRLR knockout mice may be due to a reduced production of dopamine [38]. Our present findings suggest that VDR mutant mice may have problems with endocrine control of prolactin secretion (since it is mainly controlled by inhibiting factors originated from the hypothalamus [40]).

Notably, the levels of PRL mRNA in this study were not elevated in VDR mutant mice. Finally, testing female mice and comparing their prolactin endocrinology with that of male mice may represent an interesting direction for further studies using VDR mutant mice.

In conclusion, our results suggest abnormal prolactin endocrine regulation in mice lacking functional VDR, raising the possibility that these endocrine dysfunctions may contribute to aberrant behaviours in this genetic mouse models, such as increased anxiety, specific motor deficits, stereotypic grooming and impaired nest building behaviour.

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