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Research Article

Vitamin D3-Induced Stress Response; Its Effects on Corticosterone and Hippocampal Glucocorticoid Signaling in Rats

Hira Ahmed[1]*, Sadia Gul[2], Rushda Afroz[1], Atia Gohar[2], Khurshid Jalal[2] and Darakhshan Jabeen Haleem[1,2]

[1]Neuroscience Research Laboratory, Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD), International Center for Chemical and Biological Science (ICCBS), University of Karachi, Karachi, Pakistan

[2]Neuroscience Research Laboratory, H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Science (ICCBS), University of Karachi, Karachi, Pakistan

***Corresponding Author:** Hira Ahmed, Neuroscience Research Laboratory, Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD), International Center for Chemical and Biological Science (ICCBS), University of Karachi, Karachi, Pakistan.

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Abstract

Vitamin D is a critical hormone that plays a pivotal role in various physiological processes, including bone health, calcium homeostasis, and the immune system. While vitamin D deficiency is common, excessive or prolonged intake can result in adverse effects. This study aimed to investigate the potential effects of vitamin D supplementation on locomotor activity, corticosterone levels, and glucocorticoid receptor regulation in the hippocampus. To accomplish this, male rats were randomly assigned to receive a low dose (2.5 mg/kg) and high dose (5 mg/kg) of vitamin D3 for 21 days. On day 20, open field activity was performed to assess locomotor activity. On day 21, rats were decapitated, and serum corticosterone levels were measured. Additionally, expression levels of glucocorticoid receptors in the hippocampus were analyzed using real-time PCR. Our findings suggest that vitamin D3 administration may increase stress in animals, as indicated by a reduction in exploratory activity in the open field test, an increase in serum corticosterone levels, and an upregulation of glucocorticoid receptors in the hippocampus. These results imply that vitamin D has a critical role in the regulation of the stress response and glucocorticoid signaling in the hippocampus. Moreover, caution should be exercised when determining the optimal dosage of vitamin D supplementation, as our results indicate that high doses may lead to adverse effects. In summary, our study provides new insights into the potential effects of vitamin D on the stress response and highlights the importance of carefully selecting the appropriate dose of vitamin D to avoid potential negative consequences.

Keywords: *Vitamin D; Open Field; Stress; Corticosterone; Glucocorticoid Receptor; Hippocampus*

Abbreviations

cDNA: Complementary DNA; qRT-PCR: Quantitative Real Time Polymerase Chain Reaction; GR: Glucocorticoid Receptor

Introduction

Vitamin D is commonly associated with bone health, but recent studies have focused on uncovering its metabolic processes, leading to new potential uses [1]. Research has shown that the presence of the vitamin D receptor (VDR) and activating enzyme (CYP27B1) in the

brain suggests its involvement in various brain functions [2], indicating a wider scope for its effects beyond bone health. There are several indications that vitamin D may play a role in the regulation of the stress axis [3]. The HPA axis plays a crucial role in the body's response to stress and is partly regulated by glucocorticoid receptors (GRs), which mediate the effects of cortisol, a hormone released during stress [4]. In rodents, corticosterone is the primary glucocorticoid hormone released during stress and is commonly used as a surrogate marker for cortisol in animal studies [5].

The glucocorticoid receptor (GR) serves a vital role in mediating communication between the endocrine system and the brain. Its effects on the brain can be observed during both acute and chronic stress conditions [6]. During periods of stress, the HPA axis becomes activated, resulting in elevated glucocorticoid levels and subsequent activation of the GR. This receptor is also involved in regulating behavioral responses such as mood, cognition, and emotions [7-9], as well as anxiety and depression [10,11]. It is worth noting that excessive activation of the GR may lead to mood disorders [12].

Aim of the Study

In this study, we aimed to investigate the impact of prolonged vitamin D3 administration at varying doses on exploratory behavior of rats in an open field activity as a marker for stress, as well as its effect on serum corticosterone levels and glucocorticoid receptor expression in the hippocampus.

Materials and Methods

Experimental animals

The study utilized male Albino Wistar rats weighing between 200 - 220 grams and housed in a controlled environment with a 12-hour light-dark cycle and temperature maintained at 24 ± 2°C. The rats were given free access to standard rodent food and water, and were housed and handled in accordance with the guidelines set forth in 'Guide for the care and use of laboratory animals', published by The National Academies Press, Washington D.C, USA, and the Institutional Animal Ethics Committee (IAEC) under Animal study protocol no. 2019-010. Throughout the experiment, rats were caged individually, and a one-week acclimatization period was provided for all animals.

Drug dosage

Vitamin D3, purchased from Getz Pharma, and administered via subcutaneous injection. The doses administered were 2.5 mg/kg and 5 mg/kg [13], while the control group received injections of saline (0.9% NaCl solution) [14].

Experimental protocol

The experimental animals were classified into three groups, namely the Saline treated group, the Vit D3 (2.5 mg/kg treated group), and the Vit D3 (5 mg/kg treated group), with eight animals in each group. Animals were received their respective treatments for 21 days daily between 9:15 to 9:45 am. Behavioral test was conducted using the open field activity on day 20, one hour after administering the drug. On day 21, decapitation was carried out. The hippocampus of all rats, as well as their serum, was collected and stored at -80°C for subsequent analysis.

Open field activity

The open field test is a commonly used method to assess the exploratory behavior of animals. It involves placing the animal in a square box with dimensions of 76×76 cm and walls measuring 42 cm in height. The floor of the box is divided into 25 equal squares. The animals are placed in the center of the box and their movement is observed for a period of 5 minutes. The number of squares crossed by all paws is recorded as a measure of exploratory activity. Animals displaying reduced exploratory behavior may potentially indicate anxiety or stress.

Determination of serum corticosterone

An ELISA kit manufactured by R&D Systems (catalog # KGE009) was utilized to determine the levels of corticosterone in the rat serum sample according the manufacturer's protocol.

cDNA synthesis and qRT-PCR

In the process of RNA extraction and cDNA synthesis, the samples were homogenized using trizol reagent and RNA was extracted following the manufacturer's protocol. The concentration of RNA was measured using a nanodrop spectrophotometer and the samples were stored at -80°C until further processing. The cDNA was synthesized using a commercially available kit (ThermoFisher Scientific) in accordance with the manufacturer's instructions and stored at -80°C prior to qRT-PCR analysis. For qRT-PCR, the beta-actin primer was used as a control. The mRNA levels of Glucocorticoid receptor were normalized with beta-actin mRNA. PCR was conducted using the following primers: forward (5'-ACCCACACTGTGCCCATCTA-3') and reverse (5'-CGGAACCGCTCATTGCC-3') for beta-actin and forward (5'GGTGATTGAACCCGAGGTGT-3) and reverse (5' TCACTTGACGCCCACCTAAC-3') for glucocorticoid receptor. The RT-PCR was performed using AriaMx G8830A (Agilent Technologies, USA) with Maxima SYBR Green/ROX qPCR Master Mix 2X (Thermo Scientific) according to the manufacturer's instructions. The RT-PCR parameters included denaturation at 95°C for 10 minutes, then 40 cycles at 95°C for 15 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds RT-PCR was performed using specific primers and Maxima SYBR Green/ROX qPCR Master Mix 2X, following the manufacturer's guidelines.

Statistical analysis

The statistical analysis was conducted using SPSS software version 21. The results are expressed as means ± S.D. One-way ANOVA was employed for statistical analysis, followed by Tukey's post hoc test for comparisons. A p-value of less than 0.05 was considered statistically significant.

Results

Figure 1A shows dose-related effect of vitamin D3 administration on latency to move from a central square in the open field monitored on day 20. Data analyzed via 1-way ANOVA revealed that the effect of vitamin D3 is significant ($F = 14.753$, $df = 2,21$ $p < 0.01$). According to the post hoc comparison, lower dose of vitamin D3 (2.5 mg/kg) marginally increased latency while higher dose of vitamin D3 (5 mg/kg) significantly decreased the latency, to move from the central square. Moreover, by comparing both doses, it was found that higher dose significantly decreased latency when compared with the lower dose. B) shows the dose-related effect of vitamin D3 administration in the open field activity (the number of squares crossed) monitored on day 20. Data analyzed via 1-way ANOVA revealed that the effect of vitamin D3 on number of squares crossed was significant ($F = 10.779$, $df = 2,21$ $p < 0.01$). Post-hoc test demonstrated that both doses of vitamin D3 (2.5 and 5 mg/kg) significantly decreased exploratory activity. Moreover, by comparing both doses, no difference was found between them.

Figure 2 shows dose-dependent effect of 21 days treatment of vitamin D3 on the concentration of corticosterone in serum. Analysis of data via 1-way ANOVA revealed that vitamin D3 effect was significant ($F = 4.586$, $df = 2,15$ $p < 0.05$). Post hoc test demonstrated that lower dose marginally decreased, while higher dose marginally increased the expression. Moreover, when both doses were compared, it was found that the higher dose increased corticosterone in serum as compared to lower dose.

Figure 3 shows dose-related effects of 21 days administration of vitamin D3 on glucocorticoid receptor expression in the hippocampus. Analysis of data via 1-way ANOVA revealed a significant vitamin D3 effect ($F = 36.821$, $df = 2,15$ $p < 0.01$). Post hoc test demonstrated that a slight marginal decrease was found in a lower dose while a higher dose increased the expression of the glucocorticoid receptor. Moreover, it was found that a higher dose significantly enhanced the expression as compared with lower dose.

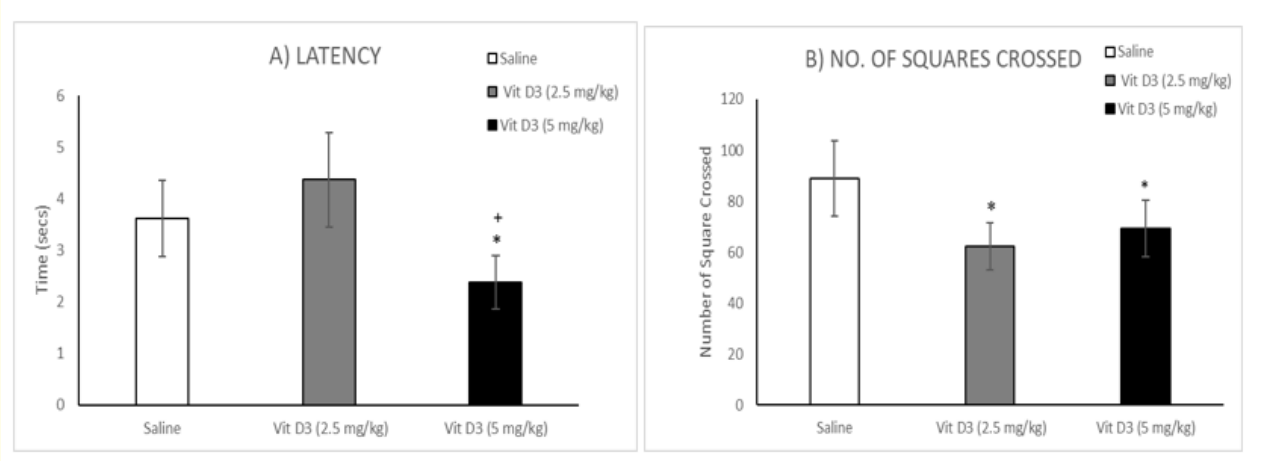

Figure 1: *Dose-dependent effects of 21 days treatment of vitamin D3 on locomotor activity in an open field A) latency to move away from central square B) number of squares crossed. Data presented as mean ± SD. Significant difference by Tukey's post-hoc comparison test: * p < 0.01 from saline-administered animals following 1-way ANOVA.*

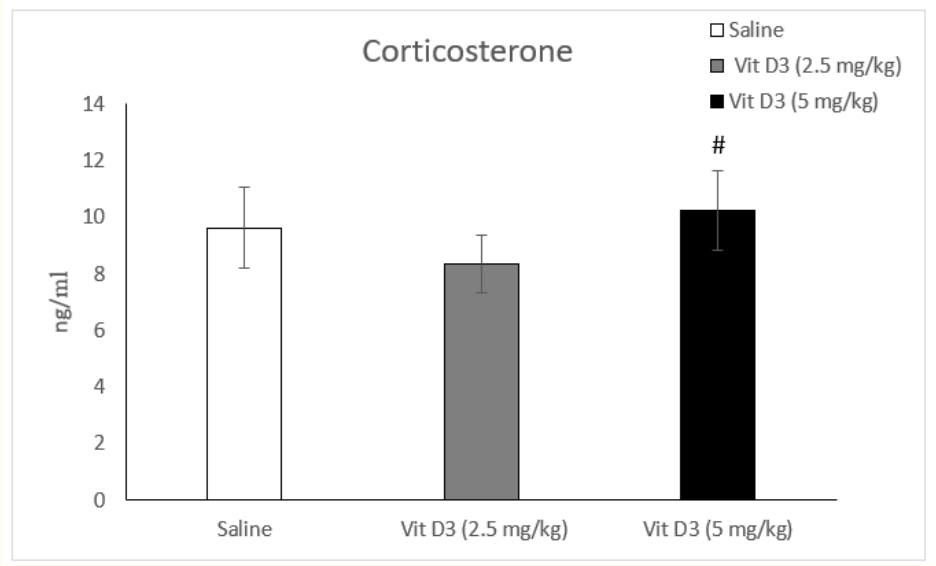

Figure 2: *Dose-related effects of 21 days administration of vitamin D3 on the concentration of corticosterone in serum. Data presented as mean ± S.D. Significant difference by Tukey's test: # p < 0.05 from vitamin D3 (2.5 mg/kg) administered animals following 1-way ANOVA.*

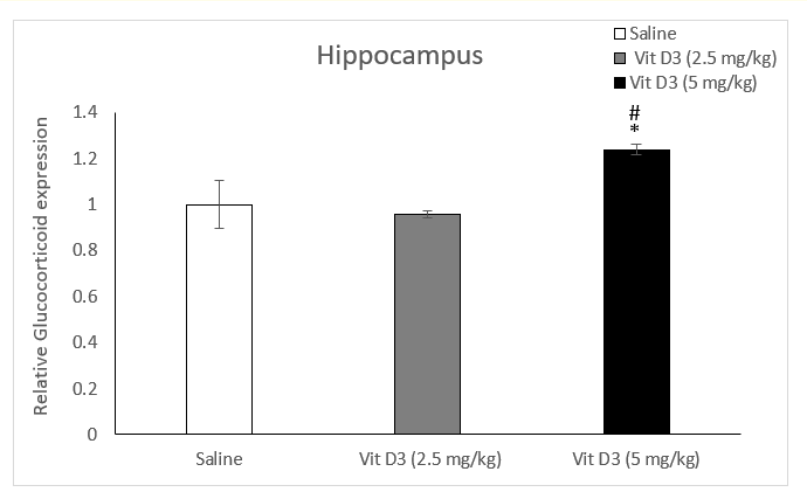

Figure 3: *Dose-dependent effects of 21 days treatment of vitamin D3 on GR expression in the hippocampus. Data presented as mean ± S.D. Significant differences by Tukey's post-hoc comparison test: * p < 0.01 from saline-administered group, #p < 0.01 from vitamin D3 (2.5 mg/kg) injected animals following 1-way ANOVA.*

Discussion

The objective of this study was to evaluate the impact of long-term administration of vitamin D3 at varying doses on the exploratory behavior of rats. The results demonstrated that both doses of vitamin D3 (2.5 and 5 mg/kg) caused a reduction in the exploratory activity of rats as evidenced by the decreased locomotor activity in an open field test, indicating a stress response. Prior research has shown that vitamin D deficiency leads to hyperlocomotion in open field [15,16]. Conversely, other studies have reported decreased locomotor activity in rats treated with 400 IU and 1000 IU doses of vitamin D [17]. In contrast, our findings suggest that supplementation at 2.5 mg/kg and 5 mg/kg of vitamin D3 causes a reduction in exploratory activity.

Furthermore, the administration of the higher dose of vitamin D3 (5 mg/kg) was found to be associated with higher levels of corticosterone, which is a hormone released in response to stress. Previous studies also indicate that vitamin D may play a role in the regulation of the stress axis [3]. In our findings, this increase in the activity of the hypothalamic-pituitary-adrenal (HPA) axis, is involved in the stress response which is also affirmed by previous researches [18]. The increase in corticosterone levels may be due to the direct effect of vitamin D3 on the adrenal gland or through the indirect effect of vitamin D3 on the HPA axis.

Moreover, the hippocampus is a region of the brain that is mainly involved in anxiety and emotions, and it has a higher number of glucocorticoid receptors [19]. Therefore, this region was selected to check the impact of vitamin D3 on glucocorticoid receptors. The administration of vitamin D3 at 5 mg/kg resulted in increased expression of glucocorticoid receptors in the hippocampus of rats. Glucocorticoid receptors are involved in the negative feedback regulation of the HPA axis, and increased expression of these receptors may indicate increased activity of the HPA axis. This finding suggests that the effect of vitamin D3 on the HPA axis is mediated, at least in part, by the increased expression of glucocorticoid receptors in the hippocampus.

To the best of our knowledge, this study represents the first investigation into the potential association between vitamin D3 and hippocampal glucocorticoid signaling in rats.

Conclusion

In conclusion, the findings of this study are important as they provide new insights into the potential effects of vitamin D supplementation on stress levels and the brain. Although vitamin D is important for maintaining bone health and immune function, excessive vitamin D intake may have harmful effects. Further studies are needed to elucidate the underlying mechanisms of the observed effects of vitamin D on HPA axis and the brain, as well as to determine the optimal dose and duration of vitamin D supplementation. Overall, this study highlights the need for caution when supplementing with vitamin D and the importance of monitoring stress levels and cognitive function in individuals receiving vitamin D supplementation.

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Conflict of Interest

The authors declare no conflict of interest.

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