Anticonvulsant effects of 1,25-dihydroxyvitamin D in chemically induced seizures in mice

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Abstract
Here, we study the role of a neurosteroid hormone Vitamin D in epilepsy. To examine this problem, we used 1,25-dihydroxyvitamin D, an active form of Vitamin D, injected subcutaneously to NMRI mice (33 μg/20 μl) 40 min prior to seizures induced by systemic injection of pentylenetetrazole (PTZ, 70 mg/kg). Overall, compared to the vehicle-treated control animals (n = 11 in each group), the Vitamin D-treated mice demonstrated reduced severity of PTZ-induced seizures (longer latency, shorter duration and lower mortality). In a separate experiment, we assessed the time-course of antiepileptic effects of 1,25-dihydroxyvitamin D. For this, we injected this compound (33 μg/20 μl) to NMRIx129S1 mice (n = 11) 40 min, 3, 6, 12 and 24 h prior to seizures, showing that antiepileptic effects were short-term, almost disappearing 3 h after administration. Our findings show that Vitamin D plays a direct anticonvulsant role in the brain and suggest that the Vitamin D endocrine system may represent a new target for the development of anticonvulsant drugs.

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1. Introduction
Numerous data indicate that the brain represents a target tissue for Vitamin D actions [4,8,21]. This steroid hormone plays an important role in the nervous system including differentiation, regulation of Ca²⁺ homeostasis, modulation of neurotransmitters release and activity of key brain genes and enzymes of neurotransmitter metabolism [4,8,31]. The functions of Vitamin D are mediated through the nuclear Vitamin D receptor (VDR), a member of the nuclear receptors superfamily of ligand-activated transcription factors [3,15]. VDR are widespread in the brain and the spinal cord, implying the potential role of both Vitamin D and VDR in the brain [4,8]. 1,25-Dihydroxyvitamin D (1,25-D, calcitriol) is the main biologically active metabolite of Vitamin D and the principal ligand for VDR, exerting its effects in a manner similar to other steroid hormones [3,8,22].

There are several lines of evidence that link various Vitamin D-related disorders to epilepsy. Low Vitamin D leads to hypocalcemia able to induce seizures due to hyperexcitability of the neural membranes [4,9]. In humans, seizures accompanied by hypocalcemia and lowered Vitamin D levels are often seen in patients with hereditary or nutritional rickets [10–14,20,24–28]. In line with this, Vitamin D and calcium therapy have long been known to reduce seizures by relieving hypocalcemia in such patients [2,9,10]. Taken together, this allowed to discuss the possibility of anticonvulsant action of Vitamin D [5], possibly due to its positive effects on mineral and hormonal homeostasis. At the same time, it has long been known that chronic treatment with antiepileptic drugs impairs mineral homeostasis in epileptic patients, leading to a marked hypocalcemia and reduced plasma levels of Vitamin D (which in turn may increase seizure) [1,6,7,12,20,23–27]. These observations have led to a wide practice of using Vitamin D as an additional therapy in epilepsy [1,6,14,23,26].

However, there is mounting evidence indicating that Vitamin D per se may play a significant role in the physiological
mechanisms underlying various brain disorders. For example, Vitamin D dysfunctions have recently been suggested to play a role in pathogenesis of multiple sclerosis, schizophrenia, anxiety and depression, rev. [4,8,15,17]. As a physiologically active neurosteroid hormone, Vitamin D is also involved in multiple neuroprotective mechanisms (rev. in [8,15,31]). Can the vitamin D system be involved in epilepsy? In addition to clinical findings described earlier, there are some animal data that directly support this notion [29,31]. In their pioneering study, Siegel et al. [29] reported direct anticonvulsant effects (increased seizure threshold in rats following the electrical stimulation of the dorsal hippocampus) within 5–10 min after i.c.v. or i.v. injection of 1,25-D. Thus, the potential role of vitamin D in epilepsy, and the possibility of direct anticonvulsant properties of this neurosteroid hormone, seem to justify further experimental investigation.

In the present study, we examined the effects of 1,25-D administration on chemically induced seizures in mice. Since pentylenetetrazole (PTZ), a potent blocker of the chloride ionophore at the gamma-aminobutyric acid GABA-A receptors, is the most frequently used chemooconvulsant in experimental models of epilepsy [16,19], we used this model in our experiments. Given a relatively high toxicity of 1,25-D due to its well-known hypercalcemic effects, we used 33 μg/20 μl of this drug in rats. Choosing the pre-treatment time for our studies, we considered earlier findings that 1,25-D may exert its anticonvulsant effects within 30–180 min in time for our studies, we considered earlier findings that 1,25-D produces a dramatic decrease in seizure latency times observed visually over a 15-min observation period and analysed by a trained observer (blind to the treatment groups) sitting in front of and 1 m away from, the testing cylinder. The latencies (s) of the first twitch, Straub tail, oro-facial, clonic and tonic seizures were analysed in both groups of mice, and reckoned as 900 s (total observation time) in the mice not showing the respective behaviours. Mortality in both groups was assessed over a 30-min period. An animal was considered dead if the heart was not beating upon manual check (the latency of death was reckoned as 1800 s if the animals remained alive after a 30-min observation period). The intensity of seizures was registered using a modified Racine’s scoring system [16]: 0 (no response), 1 (freezing), 2 (head nodding or isolated twitches), 3 (oro-facial seizure), 4 (clonic seizure), 5 (tonic seizure), 6 (death). Clonic seizures consisted of rhythmic contractions of forelimb and/or hind-limbs. Tonic seizures consisted of rigid extension of the fore- and/or hind-limbs with or without posture loss.

In a separate experiment (Experiment 2), we wanted to assess the time-course of antiepileptic effects of 1,25-D, also testing its properties in a different mouse strain. For this, we used hybrid NMRIx129S1 mice injecting them with 1,25-D (33 μg/20 μl) or vehicle 40 min, 3, 6, 12 and 24 h prior to PTZ-induced seizures (n = 11 in each group), and recording their seizure profiles as described earlier. The latencies (s) of the first twitch, oro-facial, clonic and tonic seizures were analysed in both groups of mice. Mortality in both groups was also assessed over a 30-min period.

To assess the role of Ca2+ (Experiment 3), 21 NMRIx129S1 mice were injected with the same dose of 1,25-D (40 min and 3 h prior to sampling) or vehicle (n = 7 in each group). Animals were then sacrificed and their blood samples taken to measure plasma Ca2+ levels (mmol/l) using atomic absorption spectroscopy (Yhtyneet Laboratoriot, Helsinki, Finland).

All animal care and experimental procedures in the present study were conducted in accordance with the European legislation and the guidelines of the National Institutes of Health. All animal experiments reported here were approved by the Ethical Committee of the University of Tampere. All results are expressed as mean ± S.E.M. Data were analysed by the one-way ANOVA test (Experiment 2, factor: treatment) and the Mann–Whitney U-test for independent samples (Experiments 1–3). A probability of less than 0.05 was considered statistically significant.

3. Results

The results of Experiment 1 are summarized in Table 1. Overall, 1,25-D produced a dramatic decrease in seizure
severity in PTZ-treated mice. While the duration of relatively mild oro-facial seizures was similar in both groups, the duration of more severe clonic–tonic and the total duration of seizures were markedly shorter in the mice treated with 1,25-D. For example, it is possible to assume that this steroid hormone may modulate the antiepileptic activity of 1,25-D. For example, it is possible to assume that this steroid hormone may modulate the antiepileptic activity of 1,25-D. For example, it is possible to assume that this steroid hormone may modulate the antiepileptic activity of 1,25-D. For example, it is possible to assume that this steroid hormone may modulate the antiepileptic activity of 1,25-D.

**4. Discussion**

In general, our findings demonstrate acute anticonvulsant effects of 1,25-D in the model of chemically-induced seizures in mice, and are in line with previously published studies in rats in the model of hippocampal seizures [29]. In this early study, stereotoxic injection into hippocampus or i.v. injection of 50–100 μg/kg of 1,25-D (but not Vitamin D or 25-hydroxyvitamin D) elevated seizure thresholds in rats following electrical stimulation of dorsal hippocampus—the effect lasting for 30 min (i.v.) or 180 min (i.c.v.). Explaining this and our findings, we first noted that the ability of 1,25-D to reduce seizures in the present study occurred within a relatively short (40 min) time following s.c. administration of the drug. This effect is in line with fast non-genomic action of this hormone, suggesting that its slower genomic effects may not be involved in its antiepileptic profile reported here (Table 1) and in the previously published studies [29]. Our present data extend the generality of these observations, allowing us to speculate that “fast” anticonvulsant properties of 1,25-D may represent a general pharmacological profile of this drug in different rodent models of epilepsy.

Overall, several potential mechanisms may underlie the antiepileptic activity of 1,25-D. For example, it is possible to assume that this steroid hormone may modulate the autonomic nervous system.
brain neurotransmitters and receptors (see [4,8] for details). Since GABA-A receptors represent an important target for non-genomic action of many neurosteroids and neuroactive hormones [18], it is tempting to speculate that 1,25-D may act in the brain in a similar way, modulating neuronal excitability and other neurophysiological phenomena [8]. Given the crucial role of GABAergic system in epilepsy pathogenesis, and the specific GABA-inhibiting action of PTZ, the possibility of steroid-like “fast” effects of 1,25-D on GABA-A receptors may need further experimental investigation in detail. Since 1,25-D represents a neuroactive/steroid hormone (i.e. acting and synthesised in the brain [4,15,21]), this possibility seems indeed likely.

Since numerous clinical data show anticonvulsant effects after Vitamin D therapy [1,5,6,26], it was also possible to assume that 1,25-D may affect seizures by acting via VDR to induce certain brain genes including those encoding key cytokines and enzymes of neurotransmitter metabolism [4,8]. Interestingly, for example, 1,25-D is known to down-regulate cytokines and enzymes of neurotransmitter metabolism [4,8]. This suggests that calcemic effects of this hormone may be dissociated from its antiepileptic action. In line with this notion, it was shown previously that Vitamin D treatment with 4000–16,000 IU/day led to robust clinical antiepileptic effects not related to altered plasma Ca²⁺ levels (see [1] for review). Vitamin D has also long been known to indirectly reduce the levels of Ca²⁺ in the brain (rev. in [8,15]) by stimulating the expression of several Ca-binding proteins and inhibiting the expression of L-type Ca²⁺ channels. However, our data clearly negate this hypothesis since a longer pre-treatment time with 1,25-D (needed for such effects on expression) did not reduce seizures (Table 2).

Importantly, the calcium level is not the only determining factor for the occurrence of seizures in clinic [1]. Indeed, while some seizures initially do not respond to Ca²⁺ therapy but are easily corrected with Vitamin D, individual thresholds may also be an important factor for seizure pathogenesis in humans [1] or animals [29,33]. Our present study, analysing a wide range of seizure parameters over a long period of time (Tables 1 and 2) revealed altered thresholds in mice, as assessed by their seizure latencies. It is, therefore, possible to suggest that Vitamin D, perhaps acting in a neurosteroid-like manner, may be involved in “fine tuning” of neuronal excitability, thus regulating epileptogenesis at the threshold level. In line with this hypothesis, lower circulating Vitamin D levels are reported to increase antiepileptic efficiency of 1,25-D [29]. Collectively, these data suggest possible modulation of seizure activity by Vitamin D in mice.

Comparing the data obtained in Experiments 1 and 2 (Tables 1 and 2), we note general similarity in antiepileptic effects of 33 μg 1,25-D 40 min prior to PTZ, as assessed by delayed latencies (% of the respective vehicle-treated controls, 100%): twitch (138% and 184%, NS), oro-facial (150%, P < 0.05 and 183%, NS), clonic (146%, NS and 258%, P < 0.05), tonic (107%, NS and 194%, P < 0.05) seizures and death (208%, P < 0.005 and 165%, P < 0.05). These observations further confirm robust antiepileptic effects of Vitamin D. Nonetheless, the two mouse strains used here also demonstrated some differences in their seizure profiles. For example, both NMRIx129S1 mouse groups exhibited a stronger epileptic phenotype (mortality rate 9–10/11, Experiment 2) than the NMRI groups (2–6/11, Experiment 1), also showing more individual variability (as assessed by SEM scores) in their seizure responses. These data suggest that genetic background of mice (influencing their sensitivity to PTZ, also see [33]), may also affect their responsivity to antiepileptic action of 1,25-D.

From this point of view, it may be interesting to compare several different popular mouse strains (e.g., 129S1, C57Bl6, Balb/c [33]) in their sensitivity to antiepileptic action of 1,25-D. Moreover, it may also be important to examine the role of VDR in epilepsy by analysing susceptibility to various seizures in mutant mice lacking VDR receptor gene—the animal model of Vitamin D-related rickets. These mice are currently available for biomedical research and have already been reported to show several brain dysfunctions [17]. Given the well-known link between rickets and seizures [4,10,14], testing these mice in various models of epilepsy may represent an important area of research. These studies, already underway in our laboratory, will allow to further assess the exact mechanisms of Vitamin D antiepileptic action, dissecting genomic (VDR-dependent) versus non-genomic effects of 1,25-D.

In addition, it may also be feasible to assess seizures in mice or rats chronically deprived of Vitamin D—another useful animal model of Vitamin D-related dysfunctions. Using a combination of Vitamin D deprivation with or without calcium deprivation, such studies may allow to assess the role of calcium in acute antiepileptic actions of Vitamin D. Furthermore, testing potential antiepileptic properties of other endogenous Vitamin D-related compounds (e.g., 25-hydroxyvitamin D, 24,25-dihydroxyvitamin D) may also represent an important area of epilepsy research. Collectively, these findings may enable the search for novel antiepileptic drugs based on selective non-toxic synthetic
Vitamin D-related ligands (e.g., [31]). For example, finding a steroid Vitamin D-related compound with both Vitamin D-like and GABA-modulating properties, if successful, could lead to a highly effective therapy targeting several parallel pathogenic mechanisms of epilepsy.

In summary, here we have demonstrated a clear anti-convulsant action of 1,25-D in the model of PTZ-induced seizures in mice, consistent with the previously published clinical and pre-clinical data [5,29]. In our study, 1,25-D affected predominantly the more severe stages of seizures (Figures 1 and 2). This anticonvulsant profile is clinically relevant and may be of interest for potential application. Taken together, these findings provide a neurobiological rationale for the use of Vitamin D in epilepsy—not only as a supplementary calcium-normalizing agent, but as an active antiepileptic compound per se. Overall, this study further outlines the important role of Vitamin D in the regulation of seizures, supporting the notion that the Vitamin D endocrine system may play a significant role in the physiological mechanisms underlying epilepsy [29,31].

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References