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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 pentylenenetrazole (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.

Keywords: Vitamin D; 1,25-Dihydroxyvitamin D; Epilepsy; Chemically induced seizures; Mice

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 Ca^{2+} homeostasis, modulation of neurotrophins 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

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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 chemoconvulsant 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 the drug in the present study, basing our choice on the earlier data [29] using systemic doses and injection into the brain of 50–100 μg 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 the electroconvulsive model of seizures in rats [29]. Here, we demonstrate that 1,25-D leads to reduced severity of seizures and lower mortality in the model of PTZ-induced seizures in mice.

2. Materials and methods

Subjects were 22 adult NMRI and 87 adult NMRIx129S1 mice (30–35 g; University of Tampere, Finland) maintained in a standard virus/parasite-free facility (temperature: 22 ± 2 °C; humidity: 55 ± 5%) and exposed to a 12-h light:12-h dark cycle. Lights were turned off at 18.00 and on at 06.00 h. The animals were experimentally naïve and housed in groups of five, with food and water freely available. The testing was always conducted between 14:00 and 18:00 h. On the day of the experiments, animals were transported to the experimental room and left undisturbed for 1 h for acclimation. In Experiment 1, we tested NMRI mice, a strain widely used in epilepsy research, including PTZ-induced seizures (e.g., [30,32]). 1,25-D (33 μg/20 μl (Leo Pharma, Denmark); 4 nM solution in 20% isopropanol) was injected to 11 NMRI mice s.c. using a 50 μl Hamilton syringe. Control mice ($n = 11$) received the same volume of isopropanol. After injection, the animals were returned to their cages for recovery. Forty minutes later each animal received a bolus of i.p. injection of PTZ (Sigma, UK; 70 mg/kg) and was placed into a clean glass cylinder (diameter: 20 cm; height: 30 cm) for observation of seizure profile. Between subjects, the cylinder was thoroughly cleaned with wet/dry cloths and 70% ethanol, to remove any olfactory cues.

Seizures and seizure latency times were 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 Ca^{2+} (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 Ca^{2+} 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

Table 1
Reduced susceptibility to pentylenetetrazole (70 mg/kg i.p.)-induced seizures in NMRI mice treated with 1,25-dihydroxyvitamin D (33 μg/20 μl s.c.) 40 min prior to testing

Measures	Control mice ($n = 11$)	Drug-treated mice ($n = 11$)
Latency to the first twitch (LT) (s)	56 ± 14	77 ± 25
Latency to the Straub tail (s)	51 ± 8	96 ± 24*
Latency to oro-facial seizure (LOF) (s)	62 ± 9	93 ± 15*
Latency to clonic seizure (LC) (s)	89 ± 20	130 ± 24
Latency to tonic seizure (LT) (s)	169 ± 29	181 ± 33
Latency to death (LD) (s)	839 ± 198	1743 ± 38***
Number of mice with twitches	10/11	9/11
Number of mice with Straub tail	10/11	9/11
Number of mice with oro-facial seizures	10/11	9/11
Number of mice with clonic seizures	10/11	8/11
Number of mice with tonic seizures	9/11	5/11
Mortality rate (MR)	6/11	2/11
Duration of oro-facial seizures (s)	8 ± 2	8 ± 2
Duration of clonic–tonic seizures (s)	32 ± 4	10 ± 3***
Total duration of seizures (s)	40 ± 5	18 ± 3**
Average Racine's score	5.7 ± 0.21	4.4 ± 0.40*

Data are the means ± S.E.M. Fractions represent the number of mice showing different stages of seizures (of the total number of mice in the group).
* $P < 0.05$ difference between the groups (*U*-test).
** $P < 0.01$ difference between the groups (*U*-test).
*** $P < 0.005$ difference between the groups (*U*-test).

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. As can be seen in Table 1, there was a clear tendency to longer latencies to the first twitch, Straib tail ($P < 0.05$), oro-facial ($P < 0.05$, *U*-test), clonic and tonic seizures in this group. Mortality rate was trice higher in the control group (55% versus 18%) and there was a significantly shorter latency to death compared to the 1,25-D-treated group ($P < 0.005$, *U*-test; Table 1). Finally, the average Racine's score was significantly lower in the 1,25-D-treated mice. Taken together, these data clearly indicate a robust and fast anticonvulsant action of 1,25-D in mice.

The results of Experiment 2 are summarized in Table 2. Overall, 1,25-D produced clear anticonvulsant effects in NMRIx129S1 mice if injected 40 min but not 6, 12 or 24 h prior to PTZ administration (see Table 2 for the results of data analysis using one-way ANOVA). 1,25-D (s.c. 40 min prior to PTZ) significantly delayed the onset of clonic, tonic seizures and death (Table 2). These results confirm data obtained in Experiment 1 in NMRI mice, showing similar effects of acute 1,25-D treatment in both strains. In contrast, a 3 h pre-treatment with 1,25-D produced only mild non-significant reduction of seizures in mice, compared to their vehicle-treated controls.

Finally, plasma Ca^{2+} levels were unaltered in the 3 h pre-treatment group (2.34 ± 0.06 versus 2.47 ± 0.14 mmol/l in controls, NS), showing only mild but non-significant 30% increase in the 40 min pre-treatment group (2.75 ± 0.24 mmol/l; Experiment 3).

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, stereotaxic injection into hippocampus or i.v. injection of 50–100 μg 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

Table 2
Susceptibility to pentylenetetrazole (70 mg/kg i.p.)-induced seizures in NMRIx129S1 mice treated with 1,25-dihydroxyvitamin D (33 μg/20 μl s.c.) 40 min, 3, 6, 12 and 24 h prior to testing ($n = 11$ in each group)

Mea sure	Control mice	Pre-treatment groups					
		40 min	3 h	6 h	12 h	24 h	*F*
LT	52 ± 13	96 ± 24	60 ± 15	50 ± 14	86 ± 31	58 ± 10	i
LOF	58 ± 17	106 ± 27	71 ± 16	72 ± 27	97 ± 30	73 ± 15	ii
LC	64 ± 17	165 ± 34*	78 ± 21	78 ± 16	130 ± 21	92 ± 18	iii
LT	220 ± 35	427 ± 91*	250 ± 40	209 ± 17	231 ± 46	201 ± 22	iv
LD	718 ± 159	1187 ± 153*	800 ± 162	638 ± 92	741 ± 207	515 ± 83	v
MR	10/11	9/11	11/11	10/11	9/11	11/11	

Measures as in Table 1. *F*—the results of one-way ANOVA test (factor: treatment): (i) $F_{(5,66)} = 0.99$, $P = 0.429$ (NS); (ii) $F_{(5,66)} = 0.63$, $P = 0.68$ (NS); (iii) $F(5,66) = 3.07$, $P = 0.016$ (<0.05); (iv) $F(5,66) = 3.12$, $P = 0.014$ (<0.05); (v) $F(5,66) = 2.33$, $P = 0.053$ (clear tendency).
* $P < 0.05$ difference from the control group (*U*-test).

brain neuromediators 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/neurosteroid 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 interleukin 6, recently reported to be involved in epilepsy and exert pro-convulsant effects in PTZ-induced seizures in rats [15,17]. However, the lack of anticonvulsant properties following 3–24 h pre-treatment with 1,25-D clearly negates the role of "slow" mechanisms, such as genomic effects, in this action of Vitamin D.

Since Vitamin D plays an important role in the regulation of Ca^{2+} homeostasis, another possibility for its anticonvulsant action can be altered Ca^{2+} metabolism. For example, 1,25-D has rapid effects on Ca^{2+} absorption from the intestine and other organs [12]. Therefore, it is possible to assume that 1,25-D may lead to increased plasma and reduced brain Ca^{2+} concentrations, thus contributing to overall reduction of neuronal excitation during PTZ-induced seizures. To test this hypothesis, we measured plasma Ca^{2+} levels in vehicle versus 1,25-treated mice (40 min and 3 h pre-treatment time), showing only mild non-significant transient 30% increase in plasma Ca^{2+} following 40 min, but not 3 h pre-treatment. 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 Ca^{2+} levels (see [1] for review). Finally, Vitamin D has also long been known to indirectly reduce the levels of Ca^{2+} in the brain (rev. in [8,15]) by stimulating the expression of several Ca-binding proteins and inhibiting the expression of L-type Ca^{2+} 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 Ca^{2+} 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. However, 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 did 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 anticonvulsant 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 (Tables 1 and 2), showing the anticonvulsant profile which 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

[1] F.E. Ali, M.A. Al-Bustan, W.A. Al-Busairi, F. Al-Mulla, Loss of seizure control due to anticonvulsant-induced hypocalcemia, Ann. Pharmacother. 38 (2004) 1002–1004.
[2] C. Armelisasso, M.L. Vaccario, A. Pontecorvi, S. Mazza, Tonic–clonic seizures in a patient with primary hypoparathyroidism: a case report, Clin. EEG Neurosci. 35 (2004) 97–99.
[3] L.D. Brewer, V. Thibault, K.C. Chen, M.C. Langub, P.W. Landfield, N.M. Porter, Vitamin D hormone confers neuroprotection in parallel with downregulation of L-type calcium channel expression in hippocampal neurons, J. Neurosci. 21 (2001) 98–108.
[4] S. Carswell, Vitamin D in the nervous system: actions and therapeutic potential., in: D. Feldman, F.H. Glorieux, J.W. Pike (Eds.), Vitamin D, Academic Press, San-Diego, 1997, pp. 1197–1211.
[5] C. Christiansen, P. Rorbro, O. Sjo, "Anticonvulsant" action of Vitamin D in epileptic patiens? A controlled pilot study, Br. Med. J. 2 (1974) 258–259.
[6] M.K. Drezner, Treatment of anticonvulsant drug-induced bone disease, Epilepsy Behav. 5 (2004) 41–47.
[7] L. Fitzpatrick, Pathophysiology of bone loss in patients receiving anticonvulsant therapy, Epilepsy Behav. 5 (2005) S1–S15.
[8] E. Garcion, N. Wion-Barbot, C.N. Montero-Menei, F. Berger, D. Wion, New clues about Vitamin D functions in the nervous system, Tr. Endocrinol. Metab. 13 (2002) 100–106.
[9] M.M. Gupta, D.N. Grover, Hypocalcemia and convulsions, Postgrad. Med. J. 53 (1977) 330–333.
[10] A.F. Hess, Pathogenesis of rickets, in: Rickets Including Osteomalacia and Tetany, Lea and Febiger, Philadelphia, 1929, pp. 130–142.
[11] C.C. Hoecker, J.T. Kanegaye, Recurrent febrile seizures: an unusual presentation of nutritional rickets, J. Emerg. Med. 23 (2002) 367–370.
[12] M. Holick, Stay tuned to PXR: an orphan actor that may not be D-structive only to bone, J. Clin. Invest. 115 (2005) 32–34.
[13] T. Ishikawa, K. Kawai, M. Kanayama, Y. Wada, Increased serum creatine kinase due to hypocalcemia in Vitamin D deficiency, Pediatr. Neurol. 3 (1987) 37–39.
[14] G.H. Johnson, F. Willis, Seizures as the presenting feature of rickets in an infant, Med. J. Aust. 178 (2003) 467.
[15] A.V. Kalueff, K.O. Eremin, P. Tuohimaa, Mechanisms of neuroprotective action of Vitamin D3, Biokhimia 69 (2004) 907–911.
[16] A.V. Kalueff, K. Lehtimaki, A. Ylinen, J. Honkaniemi, J. Peltola, Intranasal administration of human IL-6 increases the severity of chemically induced seizures in rats, Neurosci. Lett. (2004) 106–110.
[17] A.V. Kalueff, Y-R. Lou, I. Laaksi, P. Tuohimaa, Increased anxiety in mice lacking Vitamin D receptor gene, Neuroreport 15 (2004) 1271–1274.
[18] R. Losel, M. Wehling, Nongenomic actions of steroid hormones, Nat. Rev. 4 (2003) 46–56.
[19] A.E. Medina, A.C. Manhaes, S.L. Schmidt, Sex differences in sensitivity to seizures elicited by pentylenetetrazol in mice, Pharmacol. Biochem. Behav. 68 (2001) 591–596.
[20] M. Mikawi, R.H. Wakim, M. Fayad, Symptomatic antiepileptic drug associated Vitamin D deficiency in noninstitutionalized patients: an under-diagnosed disorder, J. Med. Liban. 51 (2003) 71–73.
[21] I. Neveu, P. Naveilhan, C. Menaa, D. Wion, P. Brachet, M. Garabedian, Synthesis of 1,25-dihydroxyvitamin D3 by rat brain macrophages in vitro, J. Neurosci. Res. 38 (1994) 214–220.
[22] A.W. Norman, W.H. Okamura, J.E. Bishop, H.L. Henry, Update on biological actions of 1alpha,25(OH)2-vitamin D3 (rapid effects) and 24R,25(OH)2-vitamin D3, Mol. Cell Endocrinol. 197 (2002) 1–13.
[23] G. Offermann, V. Pinto, R. Kruse, Antiepileptic drugs and Vitamin D supplementation, Epilepsia 20 (1979) 3–15.
[24] J. Oki, M. Takedatsu, J. Itoh, K. Yano, K. Cho, A. Okuno, Hypocalcemic focal seizures in a one-month-old infant of a mother with a low circulating level of Vitamin D, Brain Dev. 13 (1991) 132–134.
[25] A.M. Pack, Bone disease in epilepsy, Curr. Neurol. Neurosci. Rep. 4 (2004) 329–334.
[26] A.M. Pack, M.J. Morrell, Epilepsy and bone health in adults, Epilepsy Behav. 5 (2004) 24–29.
[27] J.M. Pascussi, A. Robert, M. Nguyen, O. Walrant-Debray, M. Garabedian, P. Martin, T. Pineau, J. Saric, F. Navarro, P. Maurel, M.J. Vilarem, Possible involvement of pregnane X receptor-enhanced CYP24 expression in drug-induced osteomalacia, J. Clin. Invest. 115 (2005) 177–186.
[28] I. Pela, R. Bini, D. Seracini, Vitamin D-dependent type-1 rickets: diagnosis and treatment of a further case, Pediatr. Med. Chir. 18 (1996) 207–209.
[29] A. Siegel, L. Malkowitz, M.J. Moscowitz, S. Christakos, Administration of 125-dihydroxyvitamin D3 results in the elevation of hippocampal seizure threshold levels in rats, Brain Res. 298 (1984) 125–129.
[30] M.D. Swedberg, P. Jacobsen, T. Honore, Anticonvulsant, anxiolytic and discriminative effects of the AMPA antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline (NBQX), J. Pharmacol. Exp. Ther. 274 (1995) 1113–1121.
[31] M. Tetich, M. Dziedzicka-Wasylewska, M. Kusmider, A. Kutner, M. Leskiewicz, L. Jaworska-Feil, B. Budziszewska, M. Kubera, A.M. Myint, A. Basta-Kaim, M. Skowronski, W. Lason, Effects of PRI-2191-A low-calcemic analog of 1,25-dihydroxyvitamin D(3) on the seizure-induced changes in brain gene expression and immune system activity in the rat, Brain Res. 1039 (2005) 1–13.
[32] J.M. Tusell, M. Vendrell, J. Serratosa, R. Trullas, Lindane-induced convulsions in NMRI and OF1 mice: antagonism with (+)MK-801 and voltage-dependent calcium channel blockers, Brain Res. 593 (1992) 209–214.
[33] http://aretha.jax.org/pub-cgi/phenome/mpdcgi, The Mouse Phenome Database (project: Frankel and White), The Jackson Laboratory, Bar Harbor, Maine, USA, 2001–2005.