Depression is a global problem associated with multiple social and health issues (1). Medical treatment of depression is mostly unsatisfactory, as exemplified by a recent re-analysis demonstrating the ineffectiveness of a highly promoted class of drugs used for treating depression (2). A more effective drug not belonging to the antidepressant class is expected in the near future. The relationship between depression and vitamin D has become a topic of research interest in recent years.

The likelihood of depression in subjects with vitamin D deficiency is significantly higher than in subjects who are vitamin D sufficient (3). Mean 25-hydroxyvitamin D levels were shown to be markedly lower in patients with minor and major depression than in healthy subjects (4). A clear clinical link between vitamin D and depression has not been established, however. Lansdowne and Provost investigated healthy subjects in the winter, when seasonal depression has not been established (5). A clear clinical link between vitamin D and depression (6) has not been established, however. Lansdowne and Provost investigated healthy subjects in the winter, when seasonal depression has not been established (5).

The combination of vitamin D and fluoxetine was shown to be significantly more effective for patients with major depressive disorder than fluoxetine alone (7). To date, however, no reports have been published demonstrating the effect of administration of vitamin D alone on patients with major depressive disorder. Therefore, it is necessary to examine the effect of vitamin D on the depressive state. Almost all antidepressant drugs reduce the immobility time of rats in the forced swimming test (8). Following administration, 1α-hydroxyvitamin D3 [1α(OH)D3] is metabolized in the liver to 1α,25-dihydroxyvitamin D3 [1α,25(OH)2D3], which shows an effect through 1α,25(OH)2D3 receptor (vitamin D3 receptor) distributed over the body (9) including the central nervous system (10). Therefore, we examined the effect of 1α(OH)D3 using the depression model described by Porsolt et al. (8).

Materials and Methods

A total of 40 male 5-wk-old ICR mice were obtained from Charles River Laboratory Co., Ltd. (Kyoto, Japan) 1 wk before the experiment. The mice were housed in plastic cages (3 mice/cage) with sterilized wood chips as bedding in an air-conditioned room maintained at 23±2°C and 55±5% humidity with a 12-h alternating light and dark cycle. The mice were fed a commercial pellet diet (MF, Oriental Yeast Co., Ltd., Osaka, Japan) and tap water ad libitum. The calorie sources of MF as fat and protein were 5.1 and 23.1 g/100 g, respectively. The calcium, phosphorus, and vitamin D content of MF was 1.07 g, 0.83 g, and 137 IU per 100 g, respectively. The calcium, phosphorus, and vitamin D content of MF was 1.07 g, 0.83 g, and 137 IU per 100 g, respectively. The calcium, phosphorus, and vitamin D content of MF was 1.07 g, 0.83 g, and 137 IU per 100 g, respectively. The calcium, phosphorus, and vitamin D content of MF was 1.07 g, 0.83 g, and 137 IU per 100 g, respectively. The calcium, phosphorus, and vitamin D content of MF was 1.07 g, 0.83 g, and 137 IU per 100 g, respectively. The calcium, phosphorus, and vitamin D content of MF was 1.07 g, 0.83 g, and 137 IU per 100 g, respectively. The calcium, phosphorus, and vitamin D content of MF was 1.07 g, 0.83 g, and 137 IU per 100 g, respectively.

Forced swimming tests were carried out using the method described by Porsolt et al. (8). Mice were assigned to one of four groups: Group 1 (n=10), MCT (control); Groups 2–4 (n=10 each), 1α(OH)D3. Mice were given 0.5, 1.0, or 2.0 μg/kg of 1α(OH)D3 in MCT or 0.1 mL/10 g of MCT intragastrically through a feed-

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ing tube. We gave 1α(OH)D₃ orally 24, 3, and 1 h before the forced swimming test. The mice were individually placed in a transparent cylinder (height 24 cm, diameter 10 cm) containing water at a height of 19 cm at 25±1°C. The four cylinders were partitioned off by an opaque board. The total time (in seconds) of immobility during this session was recorded and simultaneously scored for 6 min.

We examined changes in spontaneous momentum in an open field test to show that 1α(OH)D₃ differs compared with analeptic amine. We divided 40 mice into four groups and gave 1α(OH)D₃ or MCT similar to the forced swimming test before conducting the open field test. Locomotor activity was monitored for 10 min using automated Activity Monitoring Chambers (Neuroscience, Inc., Tokyo, Japan). The plastic chambers measured 23 cm (width)×40 cm (length)×18 cm (height). After recording, we analyzed the data using Excel and calculated the total movement distance of the mice as measured using a tracking system.

This study was carried out in strict accordance with the recommendations in the Guide for Animal Experiments of Okayama University Advanced Science Research Center. The protocol was approved by the Animal Care Use Committee of Okayama University Advanced Science Research Center. The protocol was approved by the Animal Care Use Committee of Okayama University Advanced Science Research Center. Special care was taken to minimize the number of animals used and their suffering in this research.

Statistical analyses. Data for all behavioral parameters are expressed as the mean±SD. When a significant difference between groups was indicated by one-way analysis of variance, we performed multiple comparisons using the Dunnett method. A p value of less than 0.05 was considered significant.

Results and Discussion

Forced swimming test

The effect of 1α(OH)D₃ on the duration of immobility is shown in Fig. 1. A dose-dependent decrease in immobility time was observed with 1α(OH)D₃ at concentrations up to 1.0 μg/kg, but there was no difference compared with the control group at a concentration of 2.0 μg/kg. At 1.0 μg/kg, 1α(OH)D₃ significantly decreased the duration of immobility as compared with the control (p<0.01).

Locomotor activity

Laboratory findings for the open field test are shown in Table 1. At all concentrations tested, 1α(OH)D₃ had no effect on locomotor activity compared with the control. Following administration, 1α(OH)D₃ is metabolized in the liver to 1α,25(OH)₂D₃, which acts hormonally through its vitamin D receptor in the body (9), including the central nervous system (10). In this study, we found that 1α,25(OH)₂D₃ decreased the immobility time of mice in the forced swimming test. Other studies have shown that the majority of antidepressants also reduce the immobility time in the forced swimming test (8, 11), as does analeptic amine (12). It has also been reported that antidepressants do not change spontaneous momentum (11), whereas analeptic amine increases spontaneous momentum (12). Because 1α(OH)D₃ did not affect the spontaneous momentum in the open field test in the present study, our results suggest that 1α(OH)D₃ may exhibit antidepressive effects. Vitamin D is reportedly effective as an adjunctive therapy with antidepressants in patients with depression (7). The results of the present study suggest that the effect of vitamin D on depression may be independent of the effect of antidepressants. At 1.0 μg/kg, 1α(OH)D₃ induced a significant reduction in immobility time (p<0.01), but the immobility time was no different than that of the controls at a concentration of 2.0 μg/kg. Threshold values for indicating a sufficient concentration of serum 25(OH)D differ in each outcome (13). The serum 1α,25(OH)₂D₃ concentration might exceed that of required levels following overdosage of 1α(OH)D₃, but this has not yet been conclusively determined. Furthermore, the expression of CYP27B1 which is a biosynthetic enzyme is inhibited in the presence of excessive active form vitamin D (14). Therefore, excess administration of 1α(OH)D₃ may stimulate a mechanism for controlling production of 1α,25(OH)₂D₃. Thus, measuring serum 1α,25(OH)₂D₃ concentrations according to 1α(OH)D₃ dose is necessary.

The hippocampal capacity of patients with unipolar depression decreases over the long term (15). The expression of hippocampal brain-derived neurotrophic factors (BDNF) also decreases in major depression, and this decrease is greatest in the hippocampus, which is related to cognitive, memory, and emotional function (16). The hippocampal capacity of patients with unipolar depression decreases over the long term (15). The expression of hippocampal brain-derived neurotrophic factors (BDNF) also decreases in major depression, and this decrease is greatest in the hippocampus, which is related to cognitive, memory, and emotional function (16). Therefore, vitamin D may have a beneficial effect on cognition, memory, and depression.

Table 1. Effect of 1α(OH)D₃ on locomotor activity.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total distance (cm) (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control (MCT: 0.1 mL/10 g)</td>
<td>442.5±83.97</td>
</tr>
<tr>
<td>2. 1α(OH)D₃ (0.5 μg/kg)</td>
<td>366.0±159.33</td>
</tr>
<tr>
<td>3. 1α(OH)D₃ (1.0 μg/kg)</td>
<td>355.2±137.31</td>
</tr>
<tr>
<td>4. 1α(OH)D₃ (2.0 μg/kg)</td>
<td>390.1±143.20</td>
</tr>
</tbody>
</table>
factor (BDNF) mRNA decreases under conditions of hippocampal atrophy (16). In addition, there is a positive association between the quantity of BDNF in the serum and cerebrocortex (17). Depression patients exhibit decreased serum levels of BDNF (18). Previous studies have shown that increased BDNF expression might play an important role in the clinical response to antidepressant therapy (19–21). Glial cell line–derived neurotrophic factor (GDNF) is a newly described member of the transforming growth factor-beta superfamily (22). Patients with major depressive disorders exhibit decreased serum GDNF levels (23). Hiasaoka et al. reported that antidepressants increase the production of GDNF in rat C6 glioma cells (C6 cells) (24). Clinically, serum GDNF increases in depressive subjects treated with antidepressants (25). These studies indicate that GDNF production by glial cells might play a role in the effects of antidepressants.

In this study, we found that 1α(OH)D₃ exerts an antidepressant-like effect in mice. 1α,25(OH)₂D₃ decreases production of BDNF induced by exercise (26), but it was shown to markedly induce expression of GDNF in vivo (27). However, our pilot study in rats showed that the levels of intracerebral BDNF and GDNF do not change after administration of vitamin D (unpublished results). Therefore, we cannot conclude that 1α,25(OH)₂D₃ exerts antidepressant activity through modulation of the expression of BDNF or GDNF. Other reports have indicated that serotonin plays a role in the effect of antidepressants based on data obtained using forced swimming tests (28, 29). As the synthesis of serotonin is activated by vitamin D (30), vitamin D might exert an antidepressant activity through modulation of the synthesis of serotonin. Further investigation is necessary to determine the precise role of vitamin D in depression.

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