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Valproic acid improves the tolerance for the stress in learned helplessness rats

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ABSTRACT

In this study, we investigated whether previously stressed rats with learned helplessness (LH) paradigm could recover from depressive-like behavior four weeks after the exposure, and also whether chronic treatment with valproic acid (VPA) could prevent behavioral despair due to the second stress on days 54 in these animals.

Four weeks after induction of LH, we confirmed behavioral remission in the previously stressed rats. Two-way analysis of variance (ANOVA) performed with two factors, pretreatment (LH or Control) and drug (VPA or Saline), revealed a significant main effect of the drug on immobility time in forced swimming test. Post hoc test showed a shorter immobility time in the LH + VPA group than in the LH + Saline group. Immunohistochemical study of synapsin I showed a significant effect of drug by pretreatment interaction on immunoreactivity of synapsin I in the hippocampus: its expression levels in the regions were higher in the LH + VPA group than in the LH + Saline group.

These results suggest that VPA could prevent the reappearance of stress-induced depressive-like behaviors in the rats recovering from prior stress, and that the drug-induced presynaptic changes in the expression of synapsin I in the hippocampus of LH animals might be related to improved tolerance toward the stress.

1. Introduction

Major depressive disorder is one of the most common and serious health problems in societies worldwide. Appropriate pharmacological or psychotherapeutic treatments are becoming accessible to increasing numbers of patients. Nevertheless, it is still associated with high rates of relapse and recurrence during a patient's lifetime. Although the average rate of recurrence in patients continuing with antidepressants was found to be 10%, their discontinuation resulted in an increase in this rate to 30% (Hirschfeld, 2001). Thus, a better understanding of the biological basis of recurrence is needed to reduce this rate.

The hippocampus is important in cognitive processes such as learning and memory (Eichenbaum et al., 1996). Several magnetic resonance imaging (MRI) studies have demonstrated decreased volume of the hippocampus in patients with major depression (Mervaala et al., 2002). Preclinical studies have shown that exposure to stress causes a range of changes in neuronal elements of this structure. These include shrinkage of apical dendrites of CA3 pyramidal neurons (Magariños et al., 1996), downregulation of neurogenesis in dentate gyrus and putative glial changes (Czéh and Lucassen, 2007 for a review). Moreover, synapse-related components in the hippocampus have also been found to be affected functionally and morphologically by stress conditions (Sousa et al., 2000; Rosenbrock et al., 2005). Antidepressants were shown to reverse some of the adverse effects of stress on the synaptic/dendritic structure in this brain region (Sairanen et al., 2007). Taken together, these results indicate enormous vulnerability to stress of the synaptic structures in the hippocampus and the ability of antidepressants to reverse the disadvantageous changes.

Learned helplessness (LH) paradigm is thought to be an animal model of depression (Seligman and Beagley, 1975). It has been well documented that LH rats present behavioral and neurochemical abnormalities that appear to be associated with depression (Martin et al., 1990). Iwata et al. (2006) showed that the expression of synapsin I (presynaptic marker) was decreased and that

Abbreviations: BDNF, brain-derived neurotrophic factor; ELISA, enzyme-linked immunosorbent assay; FST, forced swimming test; LH, learned helplessness; MAP-2, microtubule-associated protein-2; VPA, valproic acid.

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of microtubule-associated protein-2 (MAP-2, postsynaptic marker) was increased in animals about a week after the attainment of LH. In addition, Reinés et al. (2008) reported that the LH paradigm caused a reduction in the expression of synaptophysin (presynaptic marker) and postsynaptic density 95 (PSD-95: postsynaptic marker). Furthermore, antidepressants reversed both behavioral despair and their expressions to control levels, suggesting that antidepressants could alleviate behavioral and neurochemical changes in LH animals.

Valproic acid (VPA) is broadly accepted to prevent manic or depressive recurrences in patients with bipolar disorder (Gyulai et al., 2003). In addition, the drug has preclinically been shown to reduce immobility time, a key measure of behavioral despair in animal model of depression, suggesting antidepressant effects of VPA (Fernández Teruel et al., 1988). However, it is not known whether VPA could prevent the recurrence of depressive episodes in patients with unipolar depressive disorder.

In this study, we designed two-stage experimental protocols to address the recurrence-preventive effects of VPA using animals showing behavioral remission from LH. First, we produced animals with depressive-like behaviors using the LH paradigm, and then investigated whether or not such behaviors would be reduced four weeks after LH induction. We indeed confirmed the animals' behavioral improvement, which appeared to be closely related to remission in the clinical setting. Second, we administered either VPA or Saline for 21 days to those animals thereafter and assessed vulnerability to stress behaviorally using forced swimming test (FST) as well as the neurochemical changes in the hippocampus in terms of brain-derived neurotrophic factor (BDNF), presynaptic protein marker (synapsin I) and postsynaptic marker (MAP-2).

2. Experimental procedures

2.1. Animals and drugs

Animal-use procedures were in accordance with the Tottori University Guide for the Care and Use of Laboratory Animals and were approved by the Tottori University Animal Care and Use Committee. All experiments conformed to international guidelines on the ethical use of animals. The number of animals used and their suffering were minimized.

A total of 172 adult (12-week-old) male Sprague–Dawley rats weighing 250–300 g at the beginning of the experiments were used. They were housed under a 12 h light/dark cycle with free access to food and water.

VPA was kindly donated by Kyowa Hakko Kirin (Tokyo, Japan).

2.2. Experimental groups and protocol

Two-stage protocols were employed (Fig. 1). In the first stage, we produced animals with depressive-like behaviors using the LH paradigm, and then investigated whether or not such behaviors would be reduced four weeks after LH was established in advance on day 3. In order to achieve this, on day 32, we only once performed either a two-way conditioned avoidance test [LH (n = 14) and Control (n = 14)] or a FST in another set of animals [LH (n = 14) and Control (n = 14)].

The second stage was designed to identify the behavioral and neurochemical effects of the chronic treatment with VPA on LH animals. In most pharmacological experiments, dosage of 200 mg/kg, which was found to be effective in reducing immobility time in FST (Fernández Teruel et al., 1988), was given to the animals.

Twenty-eight days after the two-way conditioned avoidance test on day 3, LH and Control groups were further divided into two subgroups based on the drug treatment: one received 21 daily injections of VPA (200 mg/kg, i.p.) and the other saline for the same period of time. We thus compared the following four groups: LH + VPA, LH + Saline, Control + VPA and Control + Saline, in this study.

On the day after the last injection (on day 53), rats were fixed and their brains were removed. Immunohistochemistry for synapsin I and MAP-2 was performed as described in Section 2.6 [LH + VPA (n = 6), LH + Saline (n = 6), Control + VPA (n = 6) and Control + Saline (n = 6)]. Another set of animals were tested for BDNF, and their quantification was performed as described in Section 2.5 [LH + VPA (n = 12), LH + Saline (n = 12), Control + VPA (n = 11) and Control + Saline (n = 11)].

For FST, two swim sessions were conducted: on day 53 of the entire experimental schedule, the rats were placed in the container for 15 min, and the sessions were videotaped; on day 54, the animals were placed back into the water for 10 min. The 10 min session was subdivided into two 5 min intervals and immobility time during the first interval was measured [LH + VPA (n = 11), LH + Saline (n = 11), Control + VPA (n = 8) and Control + Saline (n = 8)].

In addition, we tested dose-dependent effects of VPA on depressive-like behaviors in FST at the three dosages of 50 mg/kg (n = 9), 100 mg/kg (n = 9) and 200 mg/kg.

2.3. Induction of depressive-like behavior by learned helplessness paradigm

Depressive-like behavior was induced by the LH paradigm according to the previous study (Shirayama et al., 2011). The LH paradigm was performed with the Gemini Avoidance System (San Diego, CA, USA). This apparatus was divided into two compartments by retractable door. On day 1 and day 2, rats were subjected to 30 inescapable electric foot shocks (0.65 mA, 30 s duration, at random intervals) in one compartment. On day 3, a two-way conditioned avoidance test was performed as a post-shock test to determine whether the rats showed the predicted escape deficits. In this study, we operationally defined escape as moving to the other compartment. Rats with 20 or more escape failures in the 30 trails were regarded as LH animals. This screening session consisted of 30 trials in which electric foot shocks [0.65 mA, 6 s duration, at random intervals (mean 30 s)] were preceded by a 3 s conditioned stimulus tone that continued until the shock was terminated. In this study, approximately 65% of the rats reached this criterion. All the rats referred to as LH animals in this study were validated individually for escape deficit.

2.4. Evaluation of the effect of VPA on forced swimming test

The effect of drugs on depressive-like behavior is usually evaluated by an avoidance task, similar to the one used in the LH paradigm. However, such a procedure was considered inappropriate in our experiments because VPA exerts an analgesic effect that could compromise the foot-shock perception by the rats, and thus may yield false-negative results in VPA-treated animals as indicated by the hot plate test (Javed et al., 2004). In this study, we therefore used FST for measurement of the depressive-like behavior.

FST was conducted as described previously (Porsolt et al., 1977). This is a standard test used to screen compounds for an antidepressant-like effect (Lucki, 1997). Swim sessions are conducted by placing rats for the time periods indicated below in plastic containers with water at a depth of 40 cm (23–25 °C), an amount deep enough so that a rat cannot touch the bottom with its hindlimbs or tail, nor can it escape. We measured immobility time during which rats made no noticeably active movements, and defined this state as depressive-like behavior.