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Research report

Microinjection of valproic acid into the ventrolateral orbital cortex exerts an antidepressant-like effect in the rat forced swim test

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ABSTRACT

Valproic acid (VPA), widely used as mood stablizer, has been shown therapeutic effects in controlling both episodes of mania and depression. The neurobiological actions by which valproic acid exerts these effects have not been established. Ventrolateral orbital cortex (VLO) is a subregion of orbitofrontal cortex (OFC) that has been closely associated with depression. However, there are few studies aimed at investigating the role of the VLO in the neurobiology of depression. In the present study, we investigated the effects on rat forced swimming test (FST) of microinjected VPA into the VLO. A single bilateral infusion of VPA into the VLO and repeated systemic administration of fluoxetine, a chemical antidepressant, significantly decreased the immobility time in the FST as compared to saline-treated controls. The effects observed in the FST paradigms could not be attributed to non-specific increases in activity since microinjection of VPA into the VLO or fluoxetine treatment did not cause general increases in locomotion test. The results provide first support for the involvement of VLO in regulating depressive-like behavior and indentify potentially important characteristics of VPA in contributing to the therapeutic action of antidepressant treatment.

1. Introduction

Affective disorders, including depression and bipolar disorder, are pervasive and devastating psychiatric disturbances that cause severe disability and economic burden [12,27]. Although significant progress has been made in the pharmacotherapy of depressive disorders, not all the sufferers respond satisfactorily to available antidepressant medications [50]. There is clearly an urgent need for the development of the treatment depressive disorders.

Valproic acid (VPA) is one of the mood stabilizers that widely used to treating bipolar disorder (BD) characterized by episodes of mania, depression and euthymia. Previous studies have shown that VPA exerts both anti-manic and antidepressant effects in treatment of BD and unipolar major depression [1,8,17]. The mechanisms by which VPA exerts its distinct effects, however, remain poorly understood. Identification of the therapeutic target of VPA will contribute to the understanding of the pathophysiology of the affective disorders and increase treatment specificity with fewer side-effects for patients.

The ventrolateral orbital cortex (VLO) has been suggested to be part of the limbic-thalamic-cortical circuits, which are highly implicated in pathogenesis of mood disorders [9,49]. However, little information is yet available on underlying neurochemical processes. GABA receptor dependent mechanisms in the VLO might be one possible neuronal substrate involved in the processes, because evidence suggests that GABAergic modulation play an important role in antidepressant treatments [20,39] and the GABAergic neurons and GABA receptors are distributed widely in the prefrontal cortex, especially VLO [15,19,29,31]. Therefore, VPA, a well known GABA enhancer [24], may exert an antidepressant effect through regulating the VLO activity. The above findings prompted the present study, aimed at testing the ability of microinjection of VPA into VLO to produce antidepressant-like activity in forced swim test (FST).

2. Material and methods

2.1. Animals

Male Sprague-Dawley rats (225–300 g) provided by the Medical Experimental Animal Center of Xi'an Jiaotong University (Shaanxi, China) were housed four per

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cage under a 12 h light/dark cycle with *ad libitum* access to food and water. The experimental protocol was approved by the Institutional Animal Care Committee of Xi'an Jiaotong University. All efforts were made to minimize the number of animals used and their suffering.

2.2. Surgical procedure

Surgery was performed in a small animal stereotaxic instrument under anesthesia with sodium pentobarbital (50 mg/kg, intraperitoneally, SCRC, Shanghai, China). For VLO infusions, bilateral stainless steel guide cannulas (0.8 mm in diameter) were inserted with their tips 2.0 mm dorsal to the VLO, at the following coordinates: 3.2 mm posterior to bregma, ±2.0 mm lateral, 4.6 mm from cortical surface (Paxinos and Watson, 1986), and attached to the skull with stainless steel screws and dental acrylic cement. Once the animals had recovered from anesthesia, they received sodium penicillin (0.2 million U/day for 5 days, intraperitoneally) to prevent wound and intracerebral infections. The animals were carefully nursed, then housed and fed in clean cages for a week recovery period. All drugs were freshly prepared before intracerebral injection.

2.3. Drug preparation and intracerebral microinjection

Fluoxetine (FLX) and sodium valproate (Sigma Chemical, St. Louis, MO, USA) were dissolved in 0.9% normal physiological saline according to required dose. For antidepressant treatment, FLX (10 mg/kg, intraperitoneally, once per day) was administrated to surgery-free rats for 14 days until 1 day before behavioral tests. For VPA infusions, microinjections were made in the postoperative rats (30 min before rats were placed back into water on test day of forced swim test or 30 min before locomotion activity test). Via the guide cannulas, VPA (100, 200 or 300 μg/0.5 μl/side) were slowly microinjected (over 60 s) into the VLO through a needle (0.4 mm in diameter) that was attached to a 1.0 μl Hamilton syringe. The needle protruded 2.0 mm beyond the guide cannula in order to approach the VLO. The same volume of vehicle (0.9% saline) was injected in the control animals.

2.4. Forced swimming test

After a week recovery period from the surgery, animals were brought to the testing room 30 min preceding the start of testing and remained in the same room throughout behavior test. The forced swim test was performed as described previously [30,41]. On the first day (pretest day), the rats are forced to swim in a plastic container (height 45 cm, diameter 25 cm) with water at a depth of 25 cm (24–26%) for 15 min. After pretest, the rats were dried immediately and returned to their home cage. On the next day (test day), the rats were placed back into the water for 10 min. The 10-min session on test day was subdivided into two 5-min intervals. Each rat's performance in the container was videotaped for scoring later. Three behaviors scored are defined as previous studies [41], climbing is defined as the movement of the rat making an active attempt to escape from the container, including searching for the escape routes and diving. Swimming is defined as the movement of the rat staying afloat or pedaling. And immobility is defined as only the movement necessary to keep their head above water.

2.5. Locomotion test

An apparatus consisted of a square Plexiglas box of 45 cm × 45 cm and 45 cm high was used to measure locomotion activity. The rats were placed in the central square of the test boxes at the beginning of the test and allowed 60 min exploration. A video-computerized tracking system (SMART, Panlab SL, Barcelona, Spain) was used to record each rat's performance and measure the distances that the rat moved.

2.6. Histology

At the end of experiments, the drug injection site was labeled with a Pontamine Sky Blue dye injection (0.5 μl, 2% in 0.5 M sodium acetate solution). Under deep anesthesia with sodium pentobarbital (100 mg/kg, intraperitoneally), the animals were transcardially perfused 100 mL of 0.01 M phosphate-buffered saline (PBS, pH 7.4), followed by 10% formalin. The brain was removed and post-fixed in 10% formalin solution for 7 days. The brain was cut in 30 μm thick sections with a freezing microtome, and the slices were stained with Cresyl Violet. The injection sites were plotted on the coronal sections modified from the Paxinos and Watson atlas (1986).

2.7. Data analysis

All data were expressed as mean ± S.E.M. One-way analyses of variance (ANOVA) were utilized to estimated the statistical differences among different treatments' effects on behavior in the forced swim test and locomotion activity test. Where appropriate, Bonferroni post hoc tests were used to determine group differences. A difference of $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Forced swimming test

The influence of bilateral microinjections of VPA (100, 200, 300 μg/0.5 μl/side) into the VLO and chronic treatment of FLX in the most commonly used model of depression, the FST, was determined. As expected, chronic FLX treatment significantly reduced the immobility time in the 10-min test compared to the control group ($p < 0.05$) (Fig. 1A). Moreover, as shown in Fig. 1A, we found that bilateral infusion of VPA (300 μg/0.5 μl/side) into the VLO also decreased the immobility time of rats in the FST ($p < 0.01$). In the other dose groups (100 and 200 μg/0.5 μl/side), VPA did not obviously influence forced swim-induced immobility over the whole 10-min session. However, bilateral microinjection of VPA with higher dose (200 and 300 μg/0.5 μl/side) into the VLO, but not the lowest dose (100 μg/0.5 μl/side), significantly decreased the immobility and increased climbing time during the first 5-min time block (Fig. 1B and C). There was no change in the duration of swimming in the FST after administration of VPA (Fig. 1D). These results suggested that VPA may exert an antidepressant-like effect in rat FST through VLO-mediated functions.

3.2. Locomotion activity

In order to examine if the reduction of immobility time in the FST was due to the general changes in activity, the influence of microinjection of VPA into VLO on locomotion activity was determined. In the locomotion test, as shown in Fig. 2A and B, there was no difference among the four treatments in "distances moved" during the first 5 min [$F(3, 24) = 0.7811$, $p > 0.05$] and 60-min test session [$F(3, 24) = 0.9903$, $p > 0.05$]. Further analyses revealed no difference between the rats groups that treated with VPA (300 μg/0.5 μl/side) and that treat with saline ($p > 0.05$). Chronic FLX treatments also produced no changes in locomotion activity compared to the control group (Fig. 2). These results indicated that the microinjection of VPA into the VLO did not significantly influence the horizontal locomotion.

3.3. Verification of microinjection sites

As shown in Fig. 3, the locations of drug injection sites were verified histologically to be within the VLO. Only animals in which syringe track was in the correct area were included in the final analyses (saline group, $n = 8$; VPA (300 μg/0.5 μl/side) group, $n = 8$; VPA (200 μg/0.5 μl/side) group, $n = 6$; VPA (100 μg/0.5 μl/side) group, $n = 6$).

4. Discussion

FST is the most widely used animal model to assess pharmacological antidepressant activity and proved to be of considerable value in elucidating pathophysiological mechanisms [30]. Pharmacological treatments clinically effective in depression are effective in reducing the amount of immobility and increase active escape behaviors, including climbing and swimming, seen in animal exposed to the uncontrollable stress [6]. The present study demonstrates that bilateral VPA microinjection into the VLO decreased the duration of the immobility time during the FST. And the treatment did not influence significantly the locomotion performance of the rats. The antidepressant effects were also seen in traditional antidepressant treatment such as fluoxetine, a kind of selective serotonin reuptake inhibitors (SSRIs). Together, our present study demonstrated that in rats, to our knowledge for the first time, microinjection of VPA into VLO produced an antidepressant-like effect.