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The voltage-gated sodium channel activator veratrine induces anxiogenic-like behaviors in rats

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HIGHLIGHTS

- Systemically administered veratrine induced anxiety-like behaviors in the rat light/dark test.
- This finding was supported in the elevated-plus maze and tail-swing behavior tests.
- Veratrine increased plasma corticosterone concentrations in rat.
- Veratrine-induced anxiety-like behaviors were abolished by riluzole and diazepam.
- This model is a novel pathological animal model for exploring possible candidate drugs for anxiolytics.

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ABSTRACT

In this study, we investigated the anxiogenic-like effects of systemically administered veratrine in rat models of anxiety. In the light/dark test, veratrine (0.6 mg/kg, s.c.) significantly and dose-dependently decreased the time rats spent in and the number of entries into a light box 30 min after administration, suggesting that veratrine increases anxiety-like behaviors. These findings were also supported by results from the elevated-plus maze test and the tail-swing behavior test. In addition, veratrine (0.6 mg/kg, s.c.) significantly increased the plasma concentration of corticosterone, an endogenous biomarker for anxiety, compared to vehicle. On the basis of these results, we conclude that veratrine induces anxiogenic-like behaviors in rats. The anxiogenic-like behaviors induced by veratrine (0.6 mg/kg, s.c.) were completely abolished by co-treatment with the typical benzodiazepine anxiolytic diazepam (1 mg/kg, s.c.), when assessed in the elevated-plus maze test. Similar results were obtained with co-treatment with riluzole (10 mg/kg, p.o.), which directly affects the glutamatergic system and has recently been suggested to have anxiolytic-like effects. In conclusion, this study provides evidence that systemically administered veratrine induces anxiogenic-like behaviors in rats. We propose the veratrine model as a novel pathological animal model to explore possible candidate drugs for anxiolytics.

1. Introduction

Dysfunction of voltage-gated sodium channels has been proposed to underlie a variety of psychiatric disorders. For example, functional alterations of SCN8A, which encodes a transmembrane protein forming the ion pore of voltage-gated sodium channels, is thought to serve as the genetic basis for psychiatric disorders such as bipolar disorder, cognitive disorders, and attempted suicide [1–3]. Anxiety symptoms are quite common and occur in many psychiatric disorders. Indeed, a preclinical study of voltage-gated sodium channel mutant mice showed that these mice exhibit heightened anxiogenic-like behaviors in the light/dark test [4]. In addition, voltage-gated sodium channel blockers have been proposed to be an effective non-benzodiazepine treatment for anxiety. For example, placebo-controlled trials showed that lamotrigine, a selective voltage-gated sodium channel blocker, was effective in treating post-traumatic stress disorder [5]. Taken together, these findings indicate that voltage-gated sodium channels could be

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promising targets for developing effective treatments for anxiety disorders.

On the other hand, growing evidence indicates that the glutamatergic system is central to the neurobiology of anxiety disorders [6]. Veratrine, a sodium channel activator, evokes presynaptic glutamate release though the opening of voltage-gated sodium channels. This notion is corroborated by the findings of a microdialysis study in rats [7–9] and a study using rat synaptosomes [10]. Recently, we also reported that the local perfusion of veratrine in the prelimbic medial prefrontal cortex of mice increased extracellular glutamate levels [11]. Interestingly, veratrine stimulated glutamate transmission and produced anxiogenic-like behaviors in mice. These anxiogenic-like behaviors were completely diminished by MK-801, a selective glutamate NMDA receptor antagonist [11]. More recently, riluzole perfusion into the prelimbic medial prefrontal cortex attenuated veratrine-evoked glutamate transmission and diminished veratrine-induced anxiogenic-like behaviors in mice open-field test [12]. However, some complicated experimental procedures limited the use of this pathological animal model as a tool to explore possible candidate drugs for novel anxiolytics.

In the present study, we therefore investigated the anxiogenic-like effects of systemically administered veratrine on rat models of anxiety. We also examined the effects of riluzole, which directly affects the glutamatergic system and has been suggested to have anxiolytic-like effects [13], on veratrine-induced anxiety-like behavior in rats.

2. Materials and methods

2.1. Animals

Male Wistar rats were used for behavioral experiments (age: 7–10 weeks; SLC, Shizuoka, Japan). The rats were housed two animals per cage in an animal room maintained at 23 ± 1 °C with a 12-h light-dark cycle (lights were automatically switched on at 8:00 am). They had free access to food and water. Rats were kept in this environment for at least one week prior to study. The study protocol was in accordance with a protocol approved by the Institutional Animal Care and Use Committee of the National Center of Neurology and Psychiatry (Approval No. 2,010,001).

2.2. Drugs

The drugs used in the present study were veratrine hydrochloride (Sigma Chemical Co., St. Louis, MO, USA); diazepam (Sigma Chemical Co.); and riluzole (RILUTEK®, 50 mg tablets; Sanofi K.K., Japan). Diazepam was used as a positive control anxiolytic drug. Veratrine hydrochloride was dissolved in saline. Diazepam was dissolved in 45% 2-hydroxypropyl-β-cyclodextrine (Wako Pure Chemical Industries, Ltd., Osaka, Japan) with saline. Riluzole tablets were crushed and suspended uniformly in 0.5% carboxymethyl cellulose (CMC). All drugs and vehicle were administered in a volume of 0.1 ml per 100 g of body weight. The dose, administration routes and pretreatment time of diazepam [14] and riluzole [15] were determined according to our previous studies.

2.3. Behavioral studies

2.3.1. Light/dark test

Testing on the light/dark test used a procedure described in our previous report [14]. Briefly, the light box apparatus consisted of two equal-sized compartments (27 cm × 23 cm × 27 cm), one light and one dark. The floors of each compartment were connected via a small opening (8.0 cm × 8.0 cm), enabling passage between the compartments. The box was elevated 70 cm above the floor and placed in indirect light (150 lx). Animals were kept in the experimental room at least 1 h for adaptation before drug administration. Diazepam and veratrine were subcutaneously administered 30 min before the test. At the beginning of the five-minute test session, each rat was placed in the dark box for one minute. The cumulative time spent in the light box and the number of entries into the light box were then registered by monitoring the rat's movements on a TV monitor attached to a video camera system (LifeCam Studio Q2F-00020). A light box visit was recorded when the rat moved at least half of its body into the light box. The observer was blinded to the treatment groups. After the removal of each animal, the apparatus was cleaned.

2.3.2. Elevated plus-maze test

Testing on the elevated plus-maze followed a procedure described in our previous report [14]. The elevated plus-maze apparatus consisted of four arms set in a cross pattern from a neutral central square. Vertical walls (closed arms, 50 cm × 10 cm × 30 cm) delimited two opposite arms, whereas the two other opposite arms had unprotected walls (open arms, 50 cm × 10 cm). To clarify the veratrine-induced anxiety-like behaviors, we set the height of the upper edge of the open arm as 1 cm. The maze was elevated 50 cm above the floor and placed in indirect light (160 lx). Animals were kept in the experimental room at least 1 h for adaptation before drug administration. Diazepam and veratrine were subcutaneously administered 30 min before the test. Riluzole was orally administered 60 min before the test. At the beginning of the five-minute test session, each rat was placed in the central zone, facing one of the open arms. The total number of visits to the closed and open arms and the cumulative time spent and visits to the open arms were then registered by monitoring the rat's movements on a TV monitor attached to a video camera system (LifeCam Studio Q2F-00020). Cumulative time was expressed as % time spent in open arms, and visits were expressed as total number of entries into the arms. An arm visit was recorded when the rat moved at least half of its body into the arm. The observer was blinded to the treatment groups. After the removal of each animal, the apparatus was cleaned.

2.3.3. Tail-swing behavior test

Testing for the tail-swing behavior used a modification of the procedure described previously [16]. Briefly, the tail-swing behavior test apparatus consisted of a stand and a rubber band hanging from a horizontal bar. The rat was suspended on the horizontal bar, which was elevated 50 cm above the floor. The apparatus was placed in indirect light (150 lx). Animals were kept in the experimental room at least 1 h for adaptation before drug administration. Diazepam and veratrine were subcutaneously administered 30 min before the test. At the beginning of the five-minute test session, each rat was suspended by rubber bands over their shoulders to force their heads upward. The cumulative time of tail-swing behavior was monitored by watching the rat's movements on a TV monitor attached to a video camera system (LifeCam Studio Q2F-00020). Tail-swing behaviors were counted when the rat swung its tail once. The observer was blinded to the treatment groups. After the removal of each animal, the apparatus was cleaned.

2.4. Corticosterone measurements

Animals were kept in the experimental room at least 1 h for adaptation before drug administration. Veratrine and vehicle were subcutaneously administered 30 min before the decapitation. The trunk blood was collected from each rat into tubes containing heparin, and these were then stored on ice. Subsequently, blood samples were centrifuged for 15 min at 3000 rpm at 4 °C. Plasma was separated immediately, aliquoted for corticosterone assay, and