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Research report

Pharmacological effect of gelsemine on anxiety-like behavior in rat

Laurence Meyer[a], Naoual Boujedaini[b], Christine Patte-Mensah[a], Ayikoe G. Mensah-Nyagan[a,*]

[a] Biopathologie de la Myéline, Neuroprotection et Stratégies Thérapeutiques, INSERM U1119, Fédération de Médecine Translationnelle de Strasbourg (FMTS), Université de Strasbourg, Bâtiment 3 de la Faculté de Médecine, 11 rue Humann, 67 000 Strasbourg, France
[b] Laboratoires BOIRON, Sainte-Foy-lès-Lyon, France

HIGHLIGHTS

- Gelsemine increased dose-dependently the number of entries in the open arms.
- Gelsemine also increased the percent of time spent in the open arms.
- Gelsemine decreased the percent of protected stretched attend postures.
- Gelsemine treatment did not affect the rat general activity.

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ABSTRACT

The chemical compound gelsemine is the major active principle of the yellow jasmine (*Gelsemium*) that is generally claimed as possessing anxiolytic properties based on empirical and indirect knowledge. Surprisingly, gelsemine effect on anxiety has until now received only little attention. Here, we used the well-validated method for anxiety assessment, the elevated plus-maze combined with video-tracking, to measure gelsemine action on rat anxiety-like behavior. Rats were intraperitoneally injected (500 μl/daily/7days) with gelsemine (10^{-6}, 10^{-10} or 10^{-14} M) or control solution. Diazepam (DZP) was used as positive standard anxiolytic and additional controls were naive rats similarly manipulated except being injected. Gelsemine or diazepam treatment did not affect the number of closed arm entries and rears illustrating the rat general activity. In contrast, gelsemine (10^{-6} to 10^{-10} M) or DZP increased dose-dependently the number of entries and the percent of time spent in the open arms indicating that gelsemine is an anxiolytic. Consistently, we observed that gelsemine (10^{-6} to 10^{-10} M) or DZP also decreased dose-dependently the percent of protected stretched attend postures, an ethological index of anxiety-like state. Altogether, our results constitute a solid set of fundamental data directly demonstrating anxiolytic properties of gelsemine. The report also opens new perspectives for the development of safe and effective gelsemine- or *Gelsemium*-based strategies against pathological anxiety.

1. Introduction

Gelsemine is an active principle and major alkaloid found in the yellow jasmine or *Gelsemium*. Chemical studies established the absolute configuration of gelsemine but its biological effects are poorly described [1–5]. In fact, preparations from *Gelsemium* are claimed to be anxiolytic drugs but, surprisingly, the action exerted by gelsemine itself on anxiety has never been investigated. Indeed, previous studies have shown that *Gelsemium* preparations may prevent stress or development of spontaneous seizures in vivo [6,7]. Recent investigations have also revealed that low doses or high dilutions of *Gelsemium*, which improve behavioral response to novel environments, induced anxiolysis in mice [8,9]. However, the anxiolytic effects of gelsemine, the main active principle of *Gelsemium*, are poorly studied and this situation does not facilitate the therapeutic exploitation of *Gelsemium* against pathological anxiety. Indeed, except a recent work which investigated on mouse anxiety-like behavior the effects of highly concentrated solutions of alkaloids isolated from Gelsemium elegans Benth [10], anxiolytic capacity of gelsemine low doses has never been studied. Furthermore, studies that investigated anxiolytic properties of *Gelsemium* have used methods such as the light–dark test and openfield which mainly assess responses to novel environments or exploratory activity but are less specific to determine accurately anxiety-related symptoms [8,9,11,12]. Therefore, we decided to perform the present study in order to evaluate the effect of different gelsemine doses on anxiety by using the elevated plus-maze apparatus coupled to video-tracking which is a well-known and

* Corresponding author. Tel.: +33 368 85 31 24; fax: +33 368 85 35 70.
E-mail address: gmensah@unistra.fr (A.G. Mensah-Nyagan).

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specific method to investigate anxiety in rodent [13–19]. In particular, we studied gelsemine effects on the number of closed arm entries considered as general activity index. Also, we assessed the action of gelsemine on various other parameters directly correlated to anxiety level including the time spent in the open arms, the percentage of open arm entries and the percent of protected stretched attend postures. To consolidate our methodological approach, we have also tested the well-known benzodiazepine diazepam as a positive standard anxiolytic drug [14,20,21].

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats weighing 250–300 g were used. The experiments were performed with male animals in order to avoid fluctuations of results intervening in females due to endogenous circulated progesterone. Animal care and manipulations were performed according to the European Community Council Directives (86/609/EC) and under the supervision of authorized investigators. All experiments were performed minimizing the number of animals used and their suffering in accordance with the Alsace Department of Veterinary Public Health Guide for the Care and Use of Laboratory Animals (Agreement number 67-186). The animals were obtained from a commercial source (Janvier, Le Genest St. Isle, France) and housed under standard laboratory conditions in a 12 h light/dark cycle with food and water ad libitum. Animals were allowed a 1 week acclimatization period before being used in experiments.

2.2. Drugs and treatment procedure

The solutions for this study were produced by Boiron Laboratories (Lyon, France) according to the European pharmacopeia. Centesimal (C) dilutions of gelsemine, starting from a hydroalcoholic solution (65% ethanol, v/v) of synthetic gelsemine (purchased from Extrasynthese, Genay France) at 1 M, were prepared in cascade with de-ionized water at 10^{-6} (3C), 10^{-10} (5C) and 10^{-14} M (7C). After each dilution step, all gelsemine solutions were successed at high speed. Control solution was prepared according to the same procedure described above, using only the hydroalcoholic solution, which was submitted to the dilution cascade with de-ionized water. All gelsemine preparations as well as control solution were kept at 4 °C before use. Animals were intraperitoneally injected daily with 500 μl of gelsemine (10^{-6}, 10^{-10} or 10^{-14} M) or with the control solution during 7 days before being behaviorally tested.

Diazepam (DZP; Roche, Neuilly-sur-Seine, France) was suspended at 5 mg/ml in a hydroalcoholic solution (5% ethanol, v/v). The control solution of diazepam (Veh-D) was prepared using only the hydroalcoholic solution (5% ethanol, v/v). Animals received a single intraperitoneal injection at 1.25 mg/kg 1 h before being behaviorally tested. Naive animals were manipulated in a similar manner without being injected. The experimenter was completely blind to the experimental conditions of the animals and to the solution contents.

2.3. Elevated plus-maze apparatus

The elevated plus-maze (EPM) consisted of two opposed open arms measuring 50 cm × 10 cm, crossed at right angle with two opposed closed arms of the same size. The latter were enclosed by walls 40 cm high, except for the entrance. The four arms delimited a central area of 10 cm^2 (Stoelting Co., Wood Dale, IL, USA). The whole maze was elevated 40 cm above the floor. The experimental sessions were monitored and recorded by a vertically mounted camera. All sessions were conducted with the experimenter present in the room. After each animal, the apparatus was cleaned with a 70% ethanol solution and totally dried before the next animal was tested.

2.4. Testing procedure

Animals were tested directly from their home cage after at least 15 min of habituation in the test room. At the start of the EPM session, each subject was placed on the central platform facing a closed arm and its behavior on the maze videotaped for 5 min. Behavioral measures were automatically scored using ANY-Maze software (Stoelting Co., Wood Dale, IL, USA). Measures scored included time spent on the open and closed arms and number of entries into the open and closed arms. Rears on the walls were also measured. An arm entry was scored when all four paws were placed in the arm, whereas an exit was considered to have taken place when at least the two front paws were placed outside of the arm. Also, stretched attend postures were measured and divided into protected and unprotected forms of these behaviors. The protected stretched attend posture was exhibited when the animal's two hind feet remained in a closed arm or the center platform while the animal elongated its head and shoulders forward, followed by subsequent retraction. Percentage of time spent on the open arms and percentage of open arm entries have repeatedly been shown to be reliable measures of anxiety on the EPM [13–18].

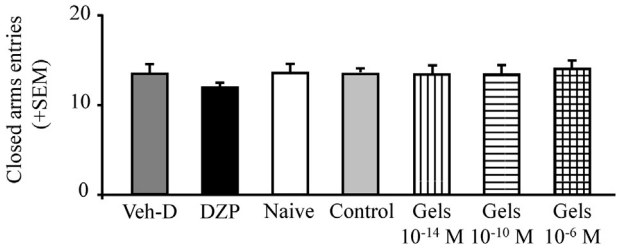

Fig. 1. Dose–response effect of gelsemine (Gels) or diazepam (DZP; 1.25 mg/kg) on the general activity index exhibited by rats in the elevated plus-maze. Each bar represents the mean (+SEM) of the number of closed arms entries observed in 12 rats per group. Veh-D: control solution of DZP; Control: control solution of Gels. Global ANOVA $F(6,77) = 0.69$, $p = 0.66$.

The measure of percent protected stretched attend postures has been suggested to be more ethologically relevant and more sensitive measures of anxiety, based on ethological analysis and pharmacological manipulations [15,22]. Because changes of motor activity may influence exploratory behavior in the elevated plus-maze, closed arm entries and rears were also measured as they are generally considered as activity and exploration indexes [14–16,23].

2.5. Statistical analysis

ANOVAs followed by Newman–Keuls post hoc comparisons were used. Data were analyzed with Statistica 9 software (Statsoft, Maisons-Alfort, France). A p-value of less than 0.05 was considered significant.

3. Results

3.1. Effects of gelsemine treatment on the exploratory and activity indexes

Fig. 1 shows the number of closed arm entries which is considered as an index of general activity. No difference was observed between naive, control- and gelsemine-treated rats indicating that gelsemine at the dose of 10^{-6}, 10^{-10} or 10^{-14} M did not modify the general activity of the animals. Similarly, no effect of gelsemine treatment was observed on the number of rears representing an index of vertical exploration (Fig. 2). Treatment of the animals with diazepam (1.25 mg/kg) or Veh-D did not affect the general activity and the number of rears (Figs. 1 and 2).

3.2. Effects of gelsemine treatment on anxiety specific parameters

The percent of time spent in the open arms (%OAT) is shown in Fig. 3. We observed that naive rats spent 10% of their time in the open arms. Injection of the control solution or the Veh-D did

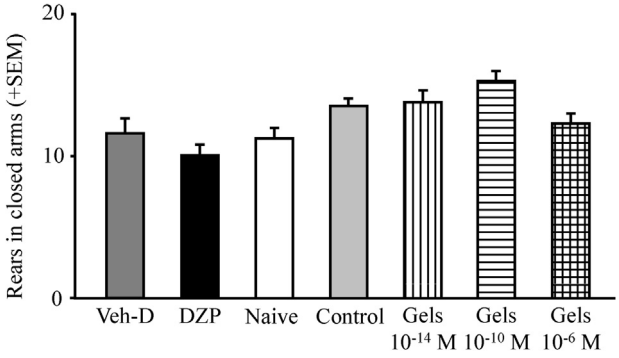

Fig. 2. Dose–response effects of gelsemine (Gels) or diazepam (DZP; 1.25 mg/kg) on the vertical exploratory activity exhibited by rats in the elevated plus-maze. Each bar is the mean (+SEM) of the number of rears on the walls of closed arms observed in 12 rats per group. Veh-D: control solution of DZP; Control: control solution of Gels. Global ANOVA $F(6,77) = 4.21$, $p < 0.05$; Newman–Keuls post hoc comparisons have showed no significant difference between groups.