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Acid-induced hyperalgesia and anxio-depressive comorbidity in rats

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HIGHLIGHTS

- Repetitive intramuscular injections of pH 4 saline cause chronic widespread hyperalgesia.
- Comorbid anxiety-like behavior exists in the acid-induced pain model.
- Comorbid depressive-like behavior exists in the acid-induced pain model.

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ABSTRACT

Fibromyalgia is a prevalent disorder characterized by chronic widespread pain (CWP) and complex comorbid symptoms. A CWP model is developed through repeated unilateral intramuscular injections of acid saline resulting in bilateral mechanical hyperalgesia in rats. The present study aims to evaluate whether both anxious and depressive comorbidities exist in this acid-induced pain model, similarly to patients with CWP syndromes. The anxiety-like behaviors were evaluated using the open field and elevated plus maze tests, and depression-like behaviors were measured by the forced swimming, sucrose consumption, and sucrose preference tests. The pain group receiving acidic saline displayed significantly lower paw withdrawal thresholds for 4 weeks than animals in the vehicle group after repetitive intramuscular injections. The pain group showed a significantly shorter duration of exploring the central zone of the open field and the open arms of the elevated plus maze compared to the vehicle group. The pain group had a significantly lower preference for and consumption of the hedonic sucrose. Moreover, rats with chronic pain showed significantly longer immobility than the vehicle group in the forced swimming test. The results indicate that psychiatric behaviors are exacerbated in the CWP model. This study provides evidence for the validity of the acid-induced pain model analogous to patients with CWP syndromes.

1. Introduction

Fibromyalgia is a chronic widespread pain (CWP) syndrome of unknown etiology characterized by widespread and long-lasting musculoskeletal pain. It affects 2% of the adult population in the United States [1]. CWP syndromes are associated with significant disability and medical costs. A considerable portion of fibromyalgia patients present with symptoms of mood disorders, particularly for anxiety and depression [2,3]. The prevalence of anxiety and depression in fibromyalgia patients are 21–64% and 30–80%, respectively [4–7]. Numerous studies also indicate that mood disorders aggravate pain syndromes and vice versa [2,8]. Chronic pain and affective disorders lead to a vicious cycle. Unfortunately, the current treatment for CWP syndromes is still unsatisfactory because of a poor understanding of the mechanisms underlying persistent pain pathways [9,10]. The available animal models play an important role in elucidating the mechanisms of the development of CWP syndromes [10,11].

An animal model with chronic muscle hyperalgesia has been developed using repeated acid injections to the gastrocnemius muscle at two- to five-day intervals to produce a long-lasting, bilateral, mechanical but not thermal hyperalgesia without motor deficits or tissue damage [12]. Morphine and pregabalin are effective to ameliorate hyperalgesia of this CWP model [13,14]. Previous studies have partially validated the acid-induced muscle pain as similar to CWP syndromes. Anxio-depressive comorbidity exists in a considerable portion of fibromyalgia patients [4–7]. Despair occurs in a CWP-like model using an amine depletion drug, reserpine [15]. However, it is unknown whether anxiety and depressive comorbidity co-occurs in the acid-induced CWP model. The present study aimed to assess the phenomena of mechanical hyperalgesia and affective behaviors after repetitive acid injections to

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validate the similarity between this acid-induced pain model and humans with CWP syndromes.

2. Materials and methods

Male Sprague–Dawley rats (10–12 weeks) were kept in a sound-attenuated room (lights on at 06:00–18:00) with food and water provided *ad libitum*. The rats were randomly assigned into a group receiving the vehicle (pH 7.2) or acidic saline (pH 4.0). The Institutional Animal Care and Use Committee of National Cheng Kung University reviewed and approved the experimental procedures. All experiments complied with the guidelines for the ethical use of animals of the US National Institutes of Health.

2.1. Induction of muscle pain

The method used to induce muscle-mediated chronic pain was performed as previously described [12]. All rats were briefly anesthetized with vaporized isoflurane (3% to 5%). After the skin covering was shaved, the left gastrocnemius muscle was injected with 100 μl neutral saline (pH 7.2) in the control group or 100 μl acidic saline (pH 4.0) in the experimental pain group on days 1 and 6. The pH of the acidic saline was adjusted with an MES acidic solution to pH 4.0 ± 0.1.

2.2. Von Frey filament testing

The rats were placed in a Lucite cubicle on an elevated metal grid allowing for the stimulation of the plantar surface of the paw. The grid hole diameter was 3 mm, and the center distance between two consecutive holes was 5 mm. The rats were placed on the platform to adapt to the apparatus for 5–10 min prior to the experimental measurement. Von Frey filaments of varying bending forces (2, 4, 6, 8, 10, 15, and 26 g) were applied to the plantar surface of the paw to assess the withdrawal response. A "response" to the stimuli was defined as an abrupt lifting of the foot upon application of the von Frey filaments. Each trial contained five von Frey stimulations, with an inter-stimulus interval of 5–6 min to reduce possible stimulus habituation. The von Frey testing forces were performed in an ascending sequence. The paw withdrawal threshold was defined as the lowest force that elicited ≥3 withdrawals in five consecutive stimulations.

The von Frey nylon hairs were calibrated both prior to and throughout the duration of the study to ensure that consistent bending forces were routinely applied. The withdrawal threshold of the ipsilateral left hindpaw was measured first, followed by the contralateral right hindpaw. The following times were tested (Fig. 1): before injection 1 (D1), 24 h after injection 1 (D2), before injection 2 (D6), 24 h after injection 2 (D7), and weekly after injection 2 over a four-week testing period (D13, D20, D27, and D34).

2.3. Body weight assessment

Body weight was assessed using a commercial scale at particular time points similar to those described for von Frey filament testing (Fig. 1).

2.4. Behavioral tests

Five behavioral tests were used to evaluate the anxiety- and depression-like behaviors of the rats [16,17]: open field, elevated plus maze, forced swimming, sucrose consumption, and sucrose preference. The open field and elevated plus maze tests were used to reveal possible signs of psychomotor disturbances that were characteristic of anxiety in an open environment. The forced swimming test was used to assess the duration of immobility, which was the experimental analog of a depressed mood; i.e., despair. The sucrose consumption test was a measure of the 'hedonic' state of an animal, or the ability to experience pleasure. Its impairment (anhedonia or decreased sensitivity to reward) was a fundamental feature of clinical depression. The sucrose preference test was performed to measure the hedonistic state of the experimental animals.

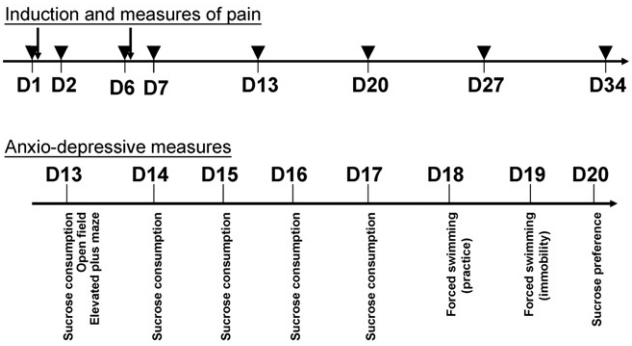

Fig. 1. Experimental procedure of the induction of chronic pain (upper panel) and anxio-depressive behavioral assessment (lower panel). The body weight and paw withdrawal threshold (▼) were measured at particular times (D1, D2, D6, D7, D13, D20, D27, D34). Vehicle (pH 7.2) or acidic (pH 4.0) saline was injected at D1 and D6 (arrows). Measures of anxiety- and depression-like behaviors were performed in particular times. The sucrose consumption test was performed for 5 days (D13–D17). The open field and elevated plus maze tests were performed at D13. Immobility of the forced swimming test was measured at D19. The sucrose preference test was performed at D20. In the first experiment, anxio-depressive behavioral measures contained the sucrose consumption, open field, elevated plus maze, and forced swimming tests. The second experiment used the sucrose preference test.

2.4.1. Elevated plus maze test

The elevated plus maze consisted of black polypropylene plastic that was elevated 68 cm above the floor. Each maze arm extended 45 cm from the junction area, which measured 13 × 13 cm. The open and closed arms were 13 cm wide, and the closed maze arms had walls extending 25 cm from the junction area. During testing, each rat was placed in the central square facing an enclosed arm and allowed to explore the maze for 10 min. The frequency of entering the open arms, the duration of time spent in the open arms, and the total movement in the elevated plus maze were analyzed. Arm entries were defined as the placement of all four paws within an arm of an elevated maze. Either the low frequency of entering the open arms or the short duration spent in the open arms is considered a validation of anxiety [18].

2.4.2. Open field test

The open field test box was composed of black acrylic plastic that formed a 99 × 99 cm square with a wall height of 45 cm. The box was divided into nine equal squares measuring 33 × 33 cm. Each rat was placed in the center zone of an open field at the beginning and allowed to explore the maze for 10 min. The frequency of crossing the central zone, the duration in the center area, and the total movement in the open field were analyzed. A low frequency of crossing the central zone or a short duration of time spent within the central zone is considered a validation of anxiety [19].

2.4.3. Sucrose consumption test

In the sucrose consumption test, each rat was placed in a test cage identical to its home cage. Consumption of a 20% sucrose solution was recorded for 15 min. The sucrose intake was measured by the volume consumed at the end of the test. Prior to each testing, the rats were not deprived of food or water. In this study, the fluid intake over 5 days was analyzed in the two groups of rats. Decreased sucrose intake, i.e., anhedonia, is a validated index of a depression-like state in animals [16,20].

2.4.4. Forced swimming test

The forced swimming test apparatus was a plastic cylinder (47 cm height, 38 cm inside diameter) containing 38 cm of water at 25 ± 1 °C. The forced swimming test consisted of two phases. In the initial