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Research report

Amitriptyline reverses hyperalgesia and improves associated mood-like disorders in a model of experimental monoarthritis

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HIGHLIGHTS

- Mood disorders in monoarthritic rats mimic those in osteoarthritic patients.
- Monoarthritic rodents display mechanical hyperalgesia and mood-like disorders.
- Amitriptyline reverses mechanical hyperalgesia in monoarthritic animals.
- Amitriptyline partly reverses depressive-like behaviour in monoarthritic animals.

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ABSTRACT

Affective disorders are common comorbidities of chronic inflammatory pain that are often overlooked in primary care. As the impact of inflammatory pain upon mood-like disorders in animal models is not well known, our objective was to assess whether prolonged experimental monoarthritis (ARTH) induced the development of anxiety and depressive-like behaviours in rodents and if amitriptyline, an antidepressant commonly used in the treatment of chronic pain, could reverse both nociceptive and mood-like impairments. Experimental ARTH was induced through an injection of kaolin/carrageenan into the right knee joint with control (SHAM) animals injected with saline. Four weeks after induction, ARTH animals displayed mechanical hyperalgesia and a depressive-like phenotype as they showed a significant increase in immobility and a decrease in the latency to immobility in the forced-swimming test at the expense of the time spent climbing/swimming. ARTH animals also displayed a decreased sucrose preference, an index of anhedonia and anxiety-like behaviour as time spent exploring the open arms of the elevated-plus-maze was decreased when compared to controls. The anxiety-like phenotype was also supported by an increase in the number of fecal boli left in the open field. In ARTH animals, the administration of amitriptyline decreased mechanical hyperalgesia and increased sucrose preference and the time spent climbing, although it had a deleterious effect in the performance of control animals. Our data show that this model of ARTH can be useful for the study of chronic pain-mood disorders comorbidities and that amitriptyline is able to partly reverse the associated nociceptive and emotional impairments.

1. Introduction

Joint pain is a condition that affects millions of people, especially the elderly, representing a huge burden on National Health Programs [1]. Pain is the most common complaint in arthritis but often patients exhibit clinical signs of comorbid depression and anxiety [2] that frequently go untreated [3].

Curiously, although many animal models of chronic inflammatory pain are available and extensively used, only a few studies addressed inflammatory pain and mood disorders comorbidity [4–7]. However, the animal models and time points chosen to perform the behavioural analysis are different among studies. For example, the time frame of osteoarthritis (OA) settlement is one feature of the behavioural experimental designs that could by itself limit the full development of neuropsychological impairments

Abbreviations: AMI, amitriptyline hydrocloride; ARTH, K/C induced monoarthritis; EPM, elevated plus maze; FC, femoral condyle; FP, femoral plateaux; FST, forced swimming test; gf, gram force; K/C, kaolin/carrageenan; LWT, limb withdrawal threshold; M, meniscus; OA, osteoarthritis; OF, open field test; PAM, pressure application measurement; PL, patellar ligament; SAL, saline; SCB, subchondral bone; SHAM, control animals; SPT, sucrose preference test; TCA, tricyclic antidepressant; TP, tibial plateau.

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(anxiety- and depressive-like behaviour) in a slow developing disease such as OA. Accordingly, recent data obtained from studies on chronic neuropathy [8] demonstrated that the experimental time frame adopted is critical to model the affective pathologies associated to chronic pain. These authors showed that anxiety and depressive-like behaviours developed in a time-dependent manner, with the former present at three weeks of neuropathy while the latter was expressed only after 6 weeks of neuropathy.

In spite of all the evidence demonstrating the existence of pain-depression comorbidity, the actual mechanisms underlying this interaction are still mostly unknown [3]. While chronic pain patients are up to four times more prone to develop major depressive disorders than the general population [9], more than half of depressed patients present comorbid chronic pain [8], with the effects of having both chronic pain and depression being worse than suffering from chronic pain or depression alone [10]. Therefore, antidepressants, including tricyclic antidepressants (TCAs), are extensively used in the management of both chronic pain and depression [11]. TCAs are the first-line drugs for the treatment of chronic pain and amitriptyline (AMI), the most commonly used TCA to treat depression in chronic pain patients [12], has also been associated with significant analgesia in different animal models of chronic neuropathic pain [13].

Accordingly, in the work herein, we used the kaolin/carrageenan model of experimental knee monoarthritis (MA) to establish a direct link between prolonged MA and the development of mood-like disorders in rodents. After four weeks of MA, we treated the animals with AMI to evaluate whether this antidepressant could reverse the nociceptive and mood-like changes induced by the model.

2. Materials and methods

2.1. Animals and ethical questions

The experiments were performed in adult male Wistar rats ($n = 44$, Charles Rivers, Barcelona, Spain) weighting 225–250 g at the beginning of the experiment. The experimental protocol was approved by the Institutional Ethical Commission and followed the European Community Council Directive 86/609/EEC and 2010/63/EU concerning the use of animals for scientific purposes. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

Animals were housed in pairs under a 12 h light cycle (starting at 8:00 a.m.) with food and water available *ad libitum*. General health parameters were surveyed twice per week by the resident veterinary and animal weight was recorded every week throughout the experimental period. Before the beginning of the experiment, all animals were handled daily by the experimenter for a week and on the day of the experimental sessions animals were left in the experimental room for an hour in order to habituate to the surroundings.

2.2. Induction of monoarthritis

The induction of arthritis (ARTH) was performed as described in detail elsewhere [14]. Briefly, 3% kaolin and 3% carrageenan (Sigma–Aldrich, St. Louis, MO, USA) were dissolved in sterile saline solution (0.9% NaCl) and injected into the synovial cavity of the right knee joint at a volume of 0.1 mL. This model produces mechanical hyperalgesia, which begins just in a few hours after surgery and lasts for several weeks [15]. Control animals (SHAM) were injected with 0.1 mL saline in the synovial cavity of the right knee joint.

2.3. Behavioural assessments

2.3.1. Nociceptive behaviour

In each animal, the development of arthritis was verified 1–2 h prior to each experiment. Only those rats that vocalized every time after five flexion–extension movements of the knee joint were considered to have developed osteoarthritis, and were included in the ARTH group. SHAM animals did not vocalize to any of the five consecutive flexion–extension movements of the knee joint.

2.3.1.1. Pressure application measurement. The application of noxious pressure to the primary site of injury is a classical approach to measure mechanical hyperalgesia [16], both in humans and animals [17]. Here, a new pressure application measurement (PAM) method was used. It allows an accurate behavioural measurement of primary mechanical hypersensitivity in rodents with chronic inflammatory joint pain [18] by the application of a force range of 0–1500 g. To perform the test and with the animal securely held, the force transducer unit (fitted to the experimenter's thumb) is placed on one side of the animal's knee joint and the forefinger on the other and an increasingly force is gradually applied across the joint until a behavioural response is observed (paw-withdrawal, freezing of whisker movement, wriggling or vocalization) with a cut-off of 5 s. The peak force (in grams force (gf)) applied immediately prior to the behavioural response is recorded as the limb withdrawal threshold (LWT). LWT was measured twice in both the ipsilateral and contralateral limbs at 1 min intervals. The mean LWTs were calculated per animal. At the end of the session animals were returned to their home cage.

2.3.2. Locomotor activity and anxious-like behaviour

2.3.2.1. Open-field test. The open field (OF) test was used to evaluate differences in the locomotor ability between SHAM and ARTH animals and followed a protocol previously implemented in our lab [19]. In summary, locomotor behaviour was assessed by measuring the total distance travelled by the animal inside the arena and anxiety-like behaviour was evaluated through the analysis of the time spent in the centre of the arena and by counting the number of faeces (*fecal boli*) remaining in the arena at the end of each trial [20]. The OF test was performed in a square arena (43.2 cm wide with the central area of the arena corresponding to a square 21.6 cm wide and equidistant from the borders) with transparent acrylic walls (Med Associates Inc., St. Albans, Vermont, USA) in a brightly illuminated room (240 lx in the centre of the arena). The test started when the animal was placed at the centre of the arena and its exploratory activity was automatically registered during 5 min. The arena was cleaned with 10% alcohol between each trial.

2.3.2.2. Elevated-plus maze. The elevated-plus maze (EPM) was used to assess anxiety-like behaviour and followed a protocol previously implemented in our lab [21]. The EPM apparatus (ENV-560; MedAssociates, St. Albans, Vermont, USA) consisted of two opposite open arms (50.8 cm × 10.2 cm) and two closed arms (50.8 cm × 10.2 cm × 40.6 cm), which were elevated 72.4 cm above the floor. At the beginning of the test the animal was placed in the centre of the maze and allowed 5 min to freely explore the maze. During the duration of the trial, behaviour was recorded through the use of an infra-red photobeam system connected to a computer with software (MedPCIV, MedAssociates) that allows for the quantification of the time spent in each arm. Between trials, the maze was cleaned with 10% ethanol. The percentage of time spent in the open arms was used as an index of anxiety-like behaviour.

2.3.3. Depressive-like behaviour

2.3.3.1. Sucrose preference test. Anhedonia, measured as a reduction in sucrose preference (SPT), was assessed through the