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Panicolytic-like effect of tramadol is mediated by opioid receptors in the dorsal periaqueductal grey

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HIGHLIGHTS

- Acute and systemic administration of tramadol causes panicolytic-like effect in the elevated T maze.
- The panicolytic-like effect of tramadol depends on activation of opioid receptors located in the dPAG.
- WAY100635 associated to tramadol promoted an anxiogenic effect in the elevated T-maze.

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ABSTRACT

Tramadol is a synthetic opioid prescribed for the treatment of moderate to severe pain, acting as agonist of μ-opioid receptors and serotonin (5-HT) and noradrenaline (NE) reuptake inhibitor. This study evaluated the effects of tramadol in rats submitted to the elevated T-maze (ETM), an animal model that evaluates behavioural parameters such as anxiety and panic. Male Wistar rats were intraperitoneally (i.p.) treated acutely with tramadol (16 and 32 mg/kg) and were submitted to the ETM. Tramadol (32 mg/kg) promoted a panicolytic-like effect. Considering that dorsal periaqueductal grey (dPAG) is the main brain structure related to the pathophysiology of panic disorder (PD), this study also evaluated the participation of 5-HT and opioid receptors located in the dPAG in the panicolytic-like effect of tramadol. Seven days after stereotaxic surgery for implantation of a cannula in the dPAG, the animals were submitted to the test. To assess the involvement of 5-HT_{1A} receptors on the effect of tramadol, we combined the 5-HT_{1A} receptor antagonist, WAY100635 (0.37 nmol), microinjected intra-dPAG, 10 min prior to the administration of tramadol (32 mg/kg, i.p.). WAY100635 did not block the panicolytic-like effect of tramadol. We also associated the non-selective opioid receptor antagonist, naloxone, systemically (1 mg/kg, i.p.) or intra-dPAG (0.5 nmol) administered 10 min prior to tramadol (32 mg/kg, i.p.). Naloxone blocked the panicolytic-like effect of tramadol in both routes of administrations, showing that tramadol modulates acute panic defensive behaviours through its interaction with opioid receptors located in the dPAG.

1. Introduction

Tramadol is a synthetic opioid with analgesic properties of central action, used in clinical practice for the treatment of moderate to severe pain of acute or chronic nature. Studies have shown that patients suffering from persistent pain have several disturbances such as difficulty falling asleep, impairment in family and social relationships, low productivity in routine tasks, functional limitation, anxiety and depression, resulting in a lower quality of life [1,2]. Its analgesic action is due to an opioid and a non-opioid mechanism, for these reasons it is considered an atypical analgesic [3]. Tramadol acts as weak agonist of μ-opioid receptors and as serotonin (5-HT) and noradrenaline (NE) reuptake inhibitor [4–6]. This monoaminergic mechanism is very similar to that of tricyclic antidepressants, and may be associated with a wide range of actions in neuropsychiatric diseases and pain management [7–9]. Tramadol has been used in non-conventional clinical practice (off-label) successfully in

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several psychiatric disorders, such as refractory major depression [10], severe suicidal ideation [11], generalized anxiety disorder, obsessive compulsive disorder and Tourette syndrome [12,13].

Panic disorder (PD) is classified as an anxiety disorder, characterized by recurrent and unexpected panic attacks, accompanied by anticipatory anxiety and/or agoraphobia, due to fear of the occurrence of further attacks. Panic attacks are responses to intense fear followed by neurovegetative symptoms such as palpitations, sweating, trembling, nausea or abdominal discomfort, pain or chest discomfort and fear of losing control. During the attack, patients have the desire to avoid or escape and a sense of death by suffocation or heart attack [14].

The neurobiological mechanism that triggers PD is based on two theories, the first related to 5-HT and the second with opioids. Deakin and Graeff (1991) theorized that the activation of the 5-HT pathway that originates from the dorsal raphe nucleus and innervates the dorsal periaqueductal grey (dPAG) inhibits the escape response to proximal danger, a behavioural response related to PD [15,16]. This theory suggests that patients with PD have a 5-HT deficit in dPAG, and an increased 5-HT action in the same region is responsible for mediating the panicolytic-like effect of antidepressant drugs [17,18]. Another study showed that administration of the antagonist of 5-HT_{1A} receptors (WAY100635) in the dPAG, blocked the panicolytic-like effect caused by chronic fluoxetine treatment in rats exposed to the elevated T-maze (ETM), suggesting an involvement of 5-HT_{1A} receptors in the panicolytic-like effect of fluoxetine [19]. The ETM is an animal model that measure two tasks in the same rat, namely, inhibitory avoidance and one-way escape, which are assumed to model anxiety and panic, respectively [20,21]. The second theory proposed by Preter and Klein (2008) is an extension of the false suffocation alarm theory that relates panic attacks to a greater sensitivity of the individual to detect increased levels of CO_2. They proposed that this system is controlled by endogenous opioids, and in PD, this mechanism is defective. This hypothesis is supported by a clinical study, which showed that naloxone administration in healthy individuals makes them sensitive to lactate-induced panic attacks, as well as in patients with PD [22,23].

Considering the interest in looking for new treatment strategies, identifying neurotransmitter systems or receptors that may be related to the etiology and, consequently, to the treatment of PD, the present article has remained focused on the panic. This study evaluated the effects of tramadol administered acutely and intraperitoneally (i.p.) in rats submitted to the ETM and its interaction with 5-HT_{1A} and opioid receptors located in the dPAG.

2. Materials and methods

2.1. Animals

Male Wistar rats, weighing 230–250 g (State University of Maringá – UEM) were kept in groups of 5 animals per cage and maintained under controlled temperature (22 ± 2 °C) and illumination (light-dark cycle of 12 h) with food and water given freely. The experiments were approved by the Ethics Committee on Animal Use of UEM (CEUA/UEM n° 1121010415).

2.2. Drugs

The drugs used in the experiments were: tramadol hydrochloride (Tramal® – racemic mixture, injectable solution 50 mg/ml; Grunenthal, Germany), naloxone hydrochloride (injectable solution 0.4 mg/ml; Hipolabor, Brazil) and WAY100635 (Sigma-Aldrich, USA). Only WAY100635 and tramadol at the dose of 16 mg/kg were diluted in 0.9% sterile saline.

2.3. Apparatus

The ETM is an apparatus made of wood, formed by three arms of equal sizes (50 × 12 cm) elevated 50 cm from the floor. One arm is enclosed by 40 cm high walls, and is perpendicular to the other two arms, which are open and opposed to each other. The open arms are protected by 1 cm high wall of acrylic (Plexiglas) transparent to avoid falls. The locomotor activity of the animals was measured in a wooden circular arena (70 cm diameter × 40 cm height).

2.4. Drug administration

Systemic administration was performed by intraperitoneal injection (1 ml/kg) of drug or saline. For intra-dPAG administration, it was inserted a needle (14 mm long and 0.3 mm outer diameter) within the guide cannula (12 mm long). The microinjections were performed with the aid of an infusion pump (Insight, Brazil), with constant time and volume (0.2 μl/120 s for WAY100635 administration and 0.5 μl/180 s for naloxone administration) using a Hamilton microsyringe of 10 μl. The needle was kept inside the cannula for 60 s after the microinjection, to prevent reflux of the injected solution and to allow a better diffusion of the drug.

2.5. Surgery

The rats were anaesthetised with an intramuscular injection of ketamine (Dopalen, Ceva, Brazil; 75 mg/kg) and xylazine (Rompun®, Bayer, Brazil; 10 mg/kg) and fixed to a stereotaxic frame (David Kopf, USA) with the bar incisor kept 2.5 mm below the interaural line. In the area of the incision was administered a subcutaneous anaesthesia with 2% lidocaine hydrochloride (Bravet, Brazil). A longitudinal incision was made to expose the skull and in the sequence, the place was cleaned to introduce a guide cannula following the rat brain atlas with reference from lambda as follows: lateral + 1.9 mm and depth – 3.2 mm with an angle of 22° [24]. The cannula was fixed to the skull with the help of an acrylic resin and two stainless-steel screws. A stainless steel-wire was inserted into the cannula, to prevent possible clogging and was removed only prior to the microinjection. After surgery, all animals received an intramuscular injection of a broad-spectrum pentabiotic combination (Pentabiótico® Veterinário 1,200,000 UI, Zoetis, Brazil; 1 ml/kg) in order to prevent possible infections in addition to a subcutaneous administration of the analgesic, anti-inflammatory and antipyretic flunixin meglumine (Banamine®, Schering-Plough Animal Health, Brazil; 2.5 ml/kg). Behavioural procedures were performed after a recovery period of 7 days.

2.6. Procedure

The rats were handled for 5 min/day on the two days that preceded the behavioural tests. Twenty-four hours before the test, we pre-exposed each animal to one of the open arms of the ETM for 30 min, making the test more sensitive because it decreases the exploration of the animal during the test [25]. The ETM test initially evaluated the profile of inhibitory avoidance, by placing each animal at the end of the closed arm, facing the intersection of the arms. The time that each animal used to leave this arm was registered in seconds (baseline). The same procedure was repeated twice at an interval of 30 s (inhibitory avoidance 1 and 2). Thirty seconds after the completion of the inhibitory avoidance tasks, each animal was placed at the end of the open arm that it was pre-exposed 24 h earlier. For this task, three consecutive trials with 30 s interval was used to assess the latency to escape from this arm with four paws (escape 1–3). The maximum time considered for both tasks was 300 s. Immediately after the ETM test, animals were placed in the circular arena for 5 min to assess the locomotor activity. The total