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Tramadol reduces anxiety-related and depression-associated behaviors presumably induced by pain in the chronic constriction injury model of neuropathic pain in rats

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ABSTRACT

Depression and anxiety are common comorbidities of neuropathic pain (NP). Pharmacological preclinical studies on NP have given abundant information on the effects of drugs on reflex measures of stimulus-evoked pain. However, few preclinical studies focus on relief of comorbidities evoked by NP. Tramadol is a weak μ-opioid receptor agonist which also inhibits the re-uptake of serotonin and norepinephrine. In this study, we investigated the effects of tramadol on nociceptive reflex, and depression-associated and anxiety-related behaviors in a NP model in rats.

We used chronic constriction injury (CCI) of the sciatic nerve as an animal model of neuropathic pain. We performed electronic von Frey tests to measure mechanical sensitivity, elevated plus maze tests (EPM) to record anxiety-related behaviors and forced swimming tests (FST) to evaluate depression-associated behaviors.

In the electronic von Frey test, CCI rats showed a decrease of 82% of the paw withdrawal threshold (PWT) compared to sham ($P < 0.001$). Tramadol increased the PWT by 336% in CCI rats ($P < 0.001$) and by 16% in sham ($P < 0.05$).

On the EPM, CCI rats spent 45% less time than sham on the open arms of the maze ($P < 0.05$). Tramadol increased the time spent on the open arms of CCI rats by 67% ($P < 0.05$) and had no significant effect on sham.

During the FST, CCI rats showed 28% longer immobility than sham ($P < 0.01$). Tramadol reduced the immobility time in CCI rats by 22% ($P < 0.001$), while having no effect on sham.

Tramadol reversed the changes in mechanical sensitivity as well as anxiety-related and depression-associated behaviors that are caused by injury of the sciatic nerve with only minor effects in the absence of injury. These data suggest that tramadol relieves chronic pain and its indirect consequences and comorbidities, and that this study also is a model for pharmacological studies seeking to investigate the effect of drugs on the major disabling symptoms of NP.

1. Introduction

Neuropathic pain is a complex clinical syndrome which affects 5% of the population in Europe, has massive costs for the health care system and is personally devastating to the people who experience it (Breivik et al., 2006). Depression and anxiety are common comorbidities of neuropathic pain, which strongly increase disabilities in patients (Asmundson and Katz, 2009; Bair et al., 2003; Kroenke et al., 2012; Verma and Gallagher, 2002).

To reduce depression and anxiety in patients suffering from neuropathic pain is a crucial treatment goal in its own right in order to increase the well-being and quality of life in these patients (Elman et al., 2011), and some neuropathic pain medications are differentially prescribed according to their efficacy on these comorbidities. However, pharmacological preclinical studies on neuropathic pain maintain their main focus on the effect of drugs on the sensitivity of animals to noxious stimuli (which mimic the so-called "evoked pain" in humans (Gregory et al., 2013)) and few preclinical studies evaluate the effects of drugs on the mood disturbances which appear when a subject has neuropathic pain.

It is difficult to address the concept of mood disturbances in rodents. However, several behavioral tests successfully use natural instincts of animals to predict the validity of treatments in human mood disturbances. The forced swimming test (FST) is based on the determination of rodents to escape an uncomfortable situation. This test can be used in preclinical studies to measure the effectiveness of antidepressants (Porsolt et al., 2001). Consequently, it is believed that this behavior in rodents is, to some level, translatable to human depression and is

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described as depression-associated behavior. Similarly, the elevated plus maze (EPM) is based on the exploration/fear conflict in rodents. This test is used in preclinical studies to predict the validity of anxiolytics in humans (Walf and Frye, 2007). Therefore, the behavior observed using EPM is termed anxiety-related behavior.

In previous studies, we showed the validity of using EPM and FST to assay anxiety-related and depression-associated behaviors in an animal model of neuropathic pain in rats (Hu et al., 2009; Roeska et al., 2008). Specifically, in one study we showed that morphine and gabapentin reversed anxiety-related behavior and mechanical hyper-nociception induced by nerve injury without affecting the anxiety-related behavior in sham operated animals. In the second study, we showed that the CB2-selective agonist, GW405833, reversed depression-associated behavior and mechanical hyper-nociception in neuropathic animals with no effects on sham animals. Together, these results suggest that the reversal of mood disturbances may be secondary to pain relief. In the present study, we used these established methods to investigate the effect of tramadol on mood disturbances in rats with chronic constriction injury (CCI).

Tramadol is a unique drug with multiple modes of action. It is a weak agonist of the μ-opioid receptor but it also inhibits the re-uptake of serotonin as well as norepinephrine. It is an analgesic and it is also considered as an antidepressant (Barber, 2011). Tramadol is effective in acute pain and in different forms of neuropathic pain and it is one of the drugs of choice in the treatment of such pain (Attal, 2012; Banerjee et al., 2013; Kroenke et al., 2009; O'Connor and Dworkin, 2009). According to the most recent meta-analysis in which efficacy and safety of different treatments were compared, tramadol has a consistent efficacy in neuropathic pain (Finnerup et al., 2010). Human pharmacokinetic studies showed that tramadol is rapidly distributed in the body and rapidly metabolized in the liver (Ardakani and Rouini, 2007). Humans and animals metabolize tramadol through the same metabolic pathway. However, the metabolization in animals is more rapid than in humans (Matthiesen et al., 1998).

The first objective of this study is to investigate the effect of tramadol on reflex measures of evoked pain as well as on depression-associated and anxiety-related behaviors. Additionally, this study strives to be an example of pharmacological preclinical studies examining symptoms of neuropathic pain, which involve the somatic as well as the affective state by using multiple outcome measures (Gregory et al., 2013).

2. Methods

2.1. Ethical statement

All the animal experiments performed in this study conformed to the German Regulations [Animal Welfare Act of 7 June 2006 (BGBl. I S. 1313). Project license AZ 35-9185.81/G-122/10 issued to Prof. Rolf-Detlef Treede by the administrative district of Karlsruhe]. Housing, handling and testing of the animals were conducted according to the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmermann, 1983). This report was written following the ARRIVE guidelines (Kilkenny et al., 2010).

2.2. Study design

We used 64 male rats divided into the following four groups (N = 16 each): sham + saline (S + S), sham + tramadol (S + T), CCI + saline (CCI + S), and CCI + tramadol (CCI + T). Each rat was tested in all three experimental conditions: EPM, electronic von Frey and FST.

The allocation of rats to the experimental groups was completed as follows. After the baseline measurements for mechanical sensitivity had been conducted, animals were randomly assigned to the sham or the CCI group. Similarly, after the surgery, the animals were randomly assigned to the saline or the tramadol treatment group. One operator performed the surgery and prepared the solutions for the injections, while a second operator performed the experimental tests. All tests were performed in a blinded manner since the experimenter was not aware of the treatment received by the rat. Additionally, the experimenter was not provided with the information about the surgery group. However, because of the difference in the posture of the paw after the CCI surgery, the two groups were easily recognizable.

We performed EPM as the first test at 25 days after surgery followed by the electronic von Frey at 28 days and the FST from 32 days on. The choice of the time points was made considering the following reasons: Anxiety-related and depression-associated behaviors do not develop in animal models of chronic pain before the third week after surgery (Andreas Kremer, personal communication) (Alba-Delgado et al., 2013; Suzuki et al., 2007). In our previous experiments we learned that in order to demonstrate anxiety-related behaviors in animal models of pain it is very important to keep the stress level of the animals to a minimum (Angelo Ceci, personal communication), we consequently choose to perform EPM as first of the three tests.

The sample size of 16 for each group was decided based on previous studies (Hu et al., 2009; Roeska et al., 2008).

The dose of 10 mg/kg tramadol was chosen because it was shown to have good efficacy on a similar animal model of neuropathic pain (Apaydin et al., 2000; Cannon et al., 2010; Guneli et al., 2007). Higher doses were not used because they have a sedative effect (Angelo Ceci, personal communication) (Cannon et al., 2010; Guneli et al., 2007).

2.3. Experimental animals and housing

Sixty-four male Wistar Han rats (Janvier, France) weighing 200–220 g at their arrival were housed in temperature-controlled standard rat individually ventilated cages (21 °C, four rats per cage). Animals were kept under the standard light cycle (6:00 a.m.–6:00 p.m.) provided with standard rodent food and tap water ad libitum. Animals were habituated to the animal room for 7 days after the delivery. The experiments were performed during day time (8:00 a.m.–3:00 p.m.).

2.4. Surgery

CCI was performed as described by Bennett and Xie (1988). In short, the left leg was shaved; the skin was incised and the muscles were separated until the nerve was visible. The adhering tissue was carefully removed, the nerve was exposed and held with curved forceps. Four loose ligatures with absorbable chromic surgical suture (4–0 USP, Resocat chrome CA365N RESORBA) were made with a distance of circa 1.0 mm. The nerve was only barely constricted. Muscle and skin were closed with absorbable suture (4–0 Vicryl, V494H, Ethicon). The sham operation was performed in the same manner until the sciatic nerve was visible without touching it. The experiments took place 25–33 days after the surgery. The animals were regularly inspected for autotomy and the body weight was monitored weekly as a physiological parameter.

2.5. Testing of sensitivity to mechanical noxious stimuli: electronic von Frey test

Sensitivity to mechanical stimuli was measured with an electronic von Frey esthesiometer equipped with a rigid cylindrical tip (diameter 0.8 mm) (Electronic von Frey esthesiometer, IITC Inc. Life Science, USA). After a minimum of 40 minute habituation in the test room, the animals were placed into a Plexiglas box (length, width, height: 20 × 10 × 14 cm) with a grid as basement for further 12 min of habituation. With the electronic esthesiometer pressure was applied on the plantar region of the right hind paw until the rat withdrew it. The paw withdrawal threshold (PWT) is calculated as the mean of five independent measurements. The baseline von Frey test was measured on two consecutive days one week after the arrival of the animals and before the surgery. The test with

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