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Research report

Opioid receptors mediate the acquisition, but not the expression of mitragynine-induced conditioned place preference in rats

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ABSTRACT

Mitragynine is the main psychoactive ingredient of the herbal drug preparation Kratom (Ketum), derived from the plant *Mitragyna speciosa.* Kratom is a widely abused drug in Southeast Asian and has a psychostimulant profile at low-medium doses, while high doses have opioidergic effects. Mitragynine was shown to possess opiate receptor affinity. However, its role in the behavioural effects of mitragynine is unclear. Here we asked whether the reinforcing effects of mitragynine are mediated by opiate receptors using a conditioned place preference (CPP) paradigm in rats. In the first experiment we tested the effects of the opiate receptor antagonist naloxone (0.1, 0.3 and 1.0 mg/kg) on the acquisition of mitragynine (10 mg/kg)-induced CPP. In the second experiment, we tested the involvement of opiate receptors in the expression of mitragynine-induced CPP in rats. We found that naloxone suppresses the acquisition of mitragynine-induced CPP. This effect was already evident at a dose of naloxone (0.1 mg/kg) which, by itself, had no conditioned place aversion (CPA) effect. Higher doses of naloxone induced a CPA and blocked mitragynine-induced CPP. In contrast, naloxone had no effect on the expression of mitragynine-induced CPP. These findings suggest that the acquisition, but not the expression of the reinforcing effects of mitragynine is mediated by opiate receptors.

1. Introduction

M. speciosa Korth (Rubiaceae), also known as ketum, kratom or biak-biak, is a traditional herbal plant indigenous to Southeast Asia [1]. To date, 44 compounds have been isolated from ketum leaves, with mitragynine being the major indole alkaloid present [2]. Mitragynine and ketum preparations are popular among local and western drug users as a substitute for opiates as well as an alternative substance for self- treatment to reduce chronic pain and opioid withdrawal symptoms [3–5]. Availability and an affordable price of the plant material contribute to its widespread use [6]. It has been reported that the regular *M. speciosa* use may lead to drug dependence with profound withdrawal symptoms, such as sleeping problems and chronic pain on the first day of abrupt drug cessation, as well as subsequent drug craving [7].

Some evidence for an addictive potential and adverse behavioral effects of mitragynine has been provided in a few animal studies [8], whereby, using a conditioned place preference (CPP) paradigm, the rewarding properties of mitragynine have been clearly demonstrated [9,10]. The rewarding properties of the drug refer to its ability to instigate and maintain the drug-related behaviours when exposed to the stimuli associated with the effects of the drug, in the absence of the drug itself [11]. Based on the previous observations that opioidergic, adrenergic, serotonergic and/or dopaminergic receptors play a role in several mitragynine's activities [8,12–14], there is a possibility that one or more receptor systems might be responsible for the mitragynine-induced CPP effects.

Thus, the present study was designed to elucidate the pharmacological mechanisms associated with mitragynine reward. Collectively, neuropharmacological studies have established that the mesocorticolimbic dopamine (DA) systems form the neurobiological basis for the rewarding properties of most abused drugs [15]. The cell bodies of the mesolimbic dopaminergic neurons are located in the ventral tegmental area (VTA) of the midbrain, which are under tonic inhibition of γ-aminobutyric acid (GABA)-ergic interneurons [16]. Opioid receptors, particularly μ-receptors, are localized on the VTA GABAergic neurons. Activation of μ-receptors leads to a decrease of GABAergic neuronal activity and consequently disinhibits DA neurons, therefore increases extracellular DA concentrations in the nucleus accumbens (NAc). Increases in the NAc DA output contribute to the expression of the

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rewarding effect of drugs of abuse [17]. In animals, the CPP effects of cocaine, amphetamine, pentobarbital and opiates were blocked by naloxone, a non-selective opioid receptor antagonist, denoting the importance of opioid receptors in motivational processes and drug reward mechanisms [18–21]. Given that mitragynine possesses high affinity towards opioid receptors [22–24], we asked whether the acquisition and/or expression of the reinforcing effects of mitragynine are mediated by the opioidergic system.

2. Material and methods

2.1. Animals

Male Sprague-Dawley rats were obtained from Animal Research and Service Centre (ARASC), Universiti Sains Malaysia, Penang, Malaysia. The rats weighed 200–300 g at the beginning of the experiment. All rats were naive and used in a single experiment only. They were socially housed in groups of six per cage under standard laboratory conditions, with temperature-controlled environment (23 ± 1 °C). The room was maintained on a 12 h light/12 h dark normal cycle (lights on from 07:00 to 19:00 h). Animals were conditioned and tested during the light phase of the cycle. Animals were handled for one week prior to commencement of the experiments with food and water available *ad libitum*. The experimental procedures were reviewed and approved by the Animal Ethics Committee of Universiti Sains Malaysia [Reference number: USM/2014/(91)(570)].

2.2. Drugs preparation

Mitragynine was extracted, isolated and verified from fresh leaves of *M. speciosa* as described previously [25]. Mitragynine (10 mg/kg) was dissolved in vehicle (20% Tween 80; Sigma-Aldrich, UK) [10] and injected i.p. immediately prior to conditioning. The dose of 10 mg/kg was selected based on previous findings that mitragynine 10 and 30 mg/kg induced a significant CPP effects [10]. Naloxone hydrochloride dihydrate (0.1, 0.3, 1.0 mg/kg; Sigma-Aldrich) was dissolved in isotonic saline (0.9% sodium chloride; A.N.B. Laboratories Co. Ltd., Thailand) and injected subcutaneously (s.c.) 15 min before conditioning or CPP test. All drug solutions were prepared immediately prior to the experiment and administered in a volume of 1 ml/kg.

2.3. Apparatus

Place conditioning was performed in a three-compartment CPP box (720 mm × 250 mm × 320 mm; TSE Place Preference System GmbH, Bad Homburg, Germany). The box consisted of two equally sized large outer chambers (305 mm × 250 mm × 320 mm; used for conditioning) separated by a small central chamber (110 mm × 250 mm × 320 mm). The two large chambers were distinct, one with gray walls (Chamber A) while the other with black walls (Chamber B). The floors in these two large chambers were different in terms of the rubber surface pattern. The overall luminosity of both chambers was similar. The central chamber had white walls and smooth Perspex floor. Each of the two separating walls of the central chamber had a door, which was opened during pre-conditioning (habituation) and CPP test days, allowing rats to enter into either of the large chambers. Infrared location sensors were mounted along the entire length of the box walls to allow monitoring of the number of entries, time spent and the distance travelled in each chamber. During conditioning and testing, the room was kept in semidarkness with minimum level of noise [10].

2.4. CPP procedures

The place conditioning procedure consisted of 3 phases: pre-conditioning, conditioning, and CPP test. Rats were weighed daily and transported to the testing room 30 min prior to the start of the experiment. A.) *Pre-conditioning (day 1–2):* During pre-conditioning phase, rats were placed into the central chamber with both doors opened to allow free access to the entire apparatus for 20 min. On day 2, the total time spent, number of entries and the distance travelled in each chamber were recorded to determine initial baseline preference of the rats. Rats which exhibited unconditioned aversion (< 10% of the session) or preferences (> 60% of the session) for any chamber were discarded for conditioning sessions. B.) *Conditioning (day 3–10)* On day 3, rats were assigned to receive either vehicle or drug paired with one of the two conditioning chambers in a counterbalanced fashion (unbiased procedure). Half of each group began the experiment with the vehicle-paired side while the other half with the drug-paired side. All rats received vehicle during pseudo-conditioning. Each rat received 4 vehicle and 4 drug pairings on alternating days, and was placed into the assigned chamber after receiving vehicle or drug injection. Each training session lasted for 1 h, once daily over 8 consecutive days. Half of each group received drug injection on the first, third, fifth and seventh day while the remaining subjects received drug injection on the second, fourth, sixth and eighth day. The central chamber was not used during conditioning and was blocked by closing the doors. Locomotor activity was measured as the distance travelled in each part of the CPP box during all conditioning- and test trials by the automatized light beam system. *C.*) *CPP test (day 11)* During the CPP test, rats were placed into the central chamber with both doors opened and were allowed free access to the entire apparatus for 20 min. The total time spent, number of entries and the distance travelled in drug-paired chamber were recorded to access individual preferences and locomotor effects of the drugs.

2.5. Experimental protocols

The experimental protocols were adapted from previous studies [18–20] with some modifications and describe in the following section.

2.5.1. Effect of naloxone on acquisition of mitragynine-induced CPP

In order to determine the effect of naloxone on the acquisition of mitragynine-induced CPP, the motivational properties of naloxone alone was first examined. Rats received either saline or naloxone (0.1, 0.3, 1.0 mg/kg) prior to vehicle administration during drug conditioning trials. On alternate days, saline injection was given prior to vehicle-paired conditioning, followed by a CPP test on day 11. To assess the effect of naloxone on the acquisition of mitragynine-induced CPP, rats were pre-treated either with saline or naloxone (0.1, 0.3, 1.0 mg/kg) prior to mitragynine administration during drug conditioning phase. On alternate days, saline injection was given prior to vehicle-paired conditioning, followed by a CPP test on day 11.

2.5.2. Effect of naloxone on expression of mitragynine-induced CPP

To assess any effect due to naloxone injected before CPP test, rats were conditioned with vehicle for both drug and vehicle conditioning, followed by saline or naloxone (0.1, 0.3, 1.0 mg/kg) administration prior to CPP test. To assess the effect of naloxone on the expression of mitragynine-induced CPP, rats were conditioned with mitragynine and vehicle during drug and vehicle conditioning phase respectively, and assigned to receive either saline or naloxone (0.1, 0.3, 1.0 mg/kg) administration prior to CPP test.

2.6. Statistical analysis

All graphical output data were expressed as mean ± SEM. Data were analysed by one-way or two-way ANOVA for repeated measures, where appropriate, followed by pre-planned Bonferroni's test. A significance level of $P < 0.05$ was used to test for statistical significance. The Prism statistical software was used to perform the statistics (version 5.01; GraphPad Software, Inc., San Diego, California USA).