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The effects of kratom on restraint–stress-induced analgesia and its mechanisms of action

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ABSTRACT

Ethnopharmacological relevance: Mitragyna speciosa and its extracts are called kratom (dried leaves, extract). They contain several alkaloids with an affinity for different opioid receptors. They are used in traditional medicine for the treatment of different diseases, as a substitute by opiate addicts, and to mitigate opioid withdrawal symptoms. Apart from their medical properties, they are used to enhance physical endurance and as a means of overcoming stress.

Purpose: The aim of this study was to determine the mechanisms underlying the effects of kratom on restraint-stress-induced analgesia which occurs during or following exposure to a stressful or fearful stimulus.

Methods: To gain further insights into the action of kratom on stress, we conducted experiments using restraint stress as a test system and stress-induced analgesia as a test parameter. Using transgenic mu opioid-receptor (MOR) deficient mice, we studied the involvement of this receptor type. We used nor-binaltorphimine (BNT), an antagonist at kappa opioid receptors (KOR), to study functions of this type of receptor. Membrane potential assay was also employed to measure the intrinsic activity of kratom in comparison to U50,488, a highly selective kappa agonist.

Results: Treatment with kratom diminished stress-induced analgesia in wildtype and MOR knockout animals. Pretreatment of MOR deficient mice with BNT resulted in similar effects. In comparison to U50,488, kratom exhibited negligible intrinsic activity at KOR alone.

Conclusions: The results suggest that the use of kratom as a pharmacological tool to mitigate withdrawal symptoms is related to its action on KOR.

1. Introduction

Mitragyna speciosa Korth. (Rubiaceae) is a tropical tree indigenous to Thailand, Malaysia, Indonesia, and Papua New Guinea (Ahmad and Aziz, 2012). More than 40 alkaloids have been isolated from its leaves (Hassan et al., 2013; Shellard, 1974). The effects of the predominant alkaloids, i.e. mitragynine, 7-hydroxymitragynine, paynantheine, speciociliatine, and peciogynine, have mainly been explained by interactions with opioidergic, adrenergic, serotonergic, and dopaminergic receptors (Babu et al., 2008; Horie et al., 2005; Matsumoto et al., 1996b; Matsumoto et al., 1996a; Shamima et al., 2012; Shellard ,1974;Shellard, 1974; Yamamoto et al., 1999; Babu et al., 2008; Stolt et al., 2014). An in vitro functional receptor assay of *Mitragyna* alkaloids revealed a partial agonist activity of (-)-mitragynine at MOR, whereas the other major alkaloids paynanthiene, speciogynine, and speciociliantine exhibited competitive antagonist activity at this receptor (Kruegel et al., 2016). The authors concluded that the gross psychoactive effects of crude plant material present a comprehensive interplay of competing agonist and antagonist effects at this opioid receptor (Kruegel et al., 2016). Beside the comprehensive interplay, the mode of action makes kratom alkaloids interesting for further research. It was reported that mitragynine and 7-hydroxymitragynine are G-protein-biased agonists of the MOR which do not recruit β-arrestin following activation (Varadi et al., 2016). It was suggested that

List of abbreviations: AI analgesic index, BNT bi-naltorphimine; CRF, corticosterone-releasing factor; DOR, delta opioid receptor; HPAA, hypothalamic-pituitary-adrenal stress axis; KO knock-out, KOR kappa opioid receptor; , WT wild type, MOR mu opioid receptor

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compounds which do not recruit β-arrestin have diminished side effects (Raehal and Bohn, 2011; Bohn, 2011; Bohn et al., 2002). Moreover, it was speculated that the pharmacophore binding of 7-hydroxymitragynine to opioid receptors is different from that of morphine (Matsumoto and Horie, 2015).

The alkaloids of kratom are used in traditional medicine to alleviate musculoskeletal pain, hypertension, coughing, and diarrhoea; to improve physical stamina and sexual performance; and to enhance physical endurance and as a means of overcoming stress. Moreover, kratom is used as a substitute for morphine in treating addicts (Apryani et al., 2010; Assanangkornchai et al., 2007; Ward et al., 2011; Singh et al., 2016). It was reported to mitigate opioid withdrawal symptoms and can induce pleasure effects (Chan et al., 2005; Hassan et al., 2013). A qualitative analysis of descriptions of human kratom use suggested that kratom could be an effective alternative to opiate drugs currently used as substitute and that it might be used by individuals to decrease symptoms of social anxiety (Swogger et al., 2015). The plant has also been used as self-medication for opiate and alcohol withdrawal (Boyer et al., 2008; Havemann-Reinecke, 2011). Actions of kratom in the central nervous system, which involve interaction with opioid receptors, descending monoaminergic projections, and neuronal Ca^{2+} channels (Matsumoto et al., 2005), might explain, at least in part, its effectiveness in reducing withdrawal symptoms.

Adverse life events and stress are considered major contributors to the initiation and continuation of addiction disorders as well as to relapse (Perreau-Lenz and Spanagel, 2015; Willner et al., 2014; Bisagno and Cadet, 2014). Evidence of the dynamic relationship between stress and opioids has been observed at the physiological level, with dysregulation of the hypothalamic-pituitary-adrenal stress axis (HPAA) in opioid addiction (Daughters et al., 2009). On the other hand, it has been reported that opiate withdrawal activates the HPAA in rats, leading to a neuronal activation of stress-related neurons in the parvocellular division of the hypothalamic paraventricular nucleus, as well as to an increase in corticosterone-releasing factor(CRF)transcription and adrenocorticotropin and corticosterone secretion (Cleck and Blendy, 2008; Navarro-Zaragoza et al., 2014). Thus drug withdrawal was considered to be a stress-like state (Chartoff and Carlezon, Jr., 2014).

One aim of addiction treatment is to reduce the severity of opiate withdrawal symptoms such as anxiety, headaches, seizures, aches, hallucinations, circulation problems, and diarrhoea. Administration of long-acting opioid agonists is the most commonly used treatment for opioid withdrawal symptoms (Kosten and O'Connor, 2003). This might be one aspect of the use of Kratom as a substitute for opiates. It binds to all types of opioid receptors (Stolt et al., 2014). However, the affinity was 150- to 300-fold lower for mu opioid receptors (MOR), about 2000-fold lower for delta opioid receptors (DOR), and 750-fold lower for kappa opioid receptors (KOR) when compared with specific agonists of these receptors. Competition experiments at the MOR and KOR for both the kratom extract and the major kratom alkaloids mitragynine and paynantheine revealed comparable affinity values (EC50), whereas mitragynine did not interact with DOR (Stolt et al., 2014).

It has been demonstrated that withdrawal from addictive drugs is accompanying with a significant stress load for addicts. Stress-induced changes results in the activation of the HPAA axis which leds to elevated synthesis and release of glucocorticoids (Matinfar et al., 2013; Morley, 1981). There is evidence that mitragynine reduced the cortisol level and the expression of stress-pathway-related genes in zebrafish undergoing a morphine withdrawal phase (Khor et al., 2011). The combination of μ opioid receptor occupation and the reduction of withdrawal symptoms might explain, at least in part, the use of kratom in addiction therapy and self-treatment. To gain further insights into the action of kratom on stress, we conducted a series of experiments using restraint stress as a test system and stress-induced analgesia as a test parameter. Stress-induced analgesia is considered a in-built mammalian pain suppression response that occurs during or following exposure to a stressful or fearful stimulus (Butler and Finn, 2009). Moreover, we studied the involvement of different opioid receptors with special regard to the MOR and the KOR. Considering the negligible DOR affinity of the kratom solution used in our experiments we did not focus on this receptor.

2. Material and methods

The work reported here was conducted in accordance with EC regulations and those of the National Act on the Use of Experimental Animals (Germany). The experimental protocol was approved by the Ethics Commission of the Federal State of Saxony-Anhalt (42502-2-1224).

2.1. Animals

To investigate the effects of kratom on stress-induced analgesia, male wild type (WT) mice (+/+) and MOR knock-out (KO, −/−) were used. The breeding of the animals (Loh et al., 1998), genotyping, 3H-[D-Ala2, N-MePhe4, Gl-ol5]-enkephalin (DAMGO) receptor autoradiography, and results of binding studies have already been published (Becker et al., 2000). After genotyping, homozygote animals were bred according to a standard breeding protocol.

The animals were kept under controlled laboratory conditions with a light/dark cycle of 12:12 (lights on at 6 a.m.), temperature 20 ± 2 °C, and air humidity 55–60%. The animals were fed with commercial pellets (ssniff R/M-H, ssniff Spezialdiäten GmbH, Soest, Germany) and tap water ad libitum. The animals were housed in groups of eight in Macrolon III cages. The mice were aged 8–9 weeks at the beginning of the experiments. Their body weight was 24–28 g. Sex-based differences in behavioural, molecular, and hormonal indices of stress in mice have been demonstrated (Butler and Finn, 2009; Litteljohn et al., 2016; Long et al., 2016). Therefore only male mice were used. All experiments were performed between 8:00 a.m. and 3:00 p.m. The animals were randomly ordered for testing to avoid the bias of circadian rhythms. In the behavioural experiments, 9–11 animals per group were used.

2.2. Vocalisation threshold in response to electrical stimulation of the tail root

This test was carried out according to the description by Charlier et al. (1961) by electrically stimulating the tail root. With the mice under etomidate anaesthesia (Hypnomidate ®, Janssen-Cilag, Neuss, Germany, 10 mg/kg), a 0.1 mm thick stainless steel wire was subcutaneously drawn through the root of the tail 72 h prior to measurement (Chindo et al., 2016). The poles completing the circuit were the metal plate in the restraining tube (2.5 cm in diameter, 9 cm long) and a terminal connected to the wire. Using an electrical stimulator (RS12, TUR, Dresden, Germany), sequences of constant incremental current (rectangular pulses, 50 ms impulse width, 50 Hz, increment 100 mA/s) were applied. The current intensity was increased constantly until the animal vocalised. The maximum stimulation was set at 300 mA (impulse peak). After vocalisation or after reaching the impulse peak, the current was immediately switched off. On test days, after habituation for 5 min in the restraining tube, pain thresholds were determined as the mean of three electrical stimulations performed at 1 min intervals. Afterwards the animals were injected with kratom or 3 v/v % ethanol as the solvent and were either returned to their home cage (non-stressed) for 3 h or they had to undergo the restraining procedure (stressed) as described below. Vocalisation threshold was then measured as described. Data was reported as basal response current (mA) and the analgesic index (AI), which was determined by accounting for each individual's basal response as well as the maximum stimulation imposed using the following calculation: AI =(vocalisation response