American Pain Society
RESEARCH
EDUCATION
TREATMENT
ADVOCACY
PUBLISHED BY ELSEVIER

The Journal of Pain, Vol 19, No 5 (May), 2018: pp 487-495
Available online at www.jpain.org and www.sciencedirect.com

The Involvement of the Endocannabinoid System in the Peripheral Antinociceptive Action of Ketamine

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Abstract: **Ketamine has been widely used as an analgesic and produces dissociative anesthetic effects. The antinociceptive effects of ketamine have been studied, but the involvement of endocannabinoids in these effects has not yet been investigated. In this study, we evaluated the involvement of the endocannabinoid system in the peripheral antinociceptive effects induced by ketamine. All drugs were administered via the intraplantar route. To induce hyperalgesia, rat paws were injected with prostaglandin E_2 (2 μg per paw). The nociceptive threshold for mechanical stimulation was measured in the right hind paw of Wistar rats using the Randall–Selitto test. The tissue levels of anandamide (AEA), 2-arachidonoylglycerol, palmitoylethanolamide, and oleoylethanolamide were measured using liquid chromatography coupled to single quadrupole mass spectrometry. The administration of the cannabinoid receptor type 1 (CB_1) antagonist, N(piperidine-1yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl 1 pyrazolcarboxamide (20, 40, and 80 μg per paw), but not the cannabinoid receptor type 2 antagonist, 6-iodo-2-methyl-1-(2-morpholinoethyl)-1H-indol-3-yl) (4-methoxyphenyl) methanone (100 μg per paw), antagonized the ketamine-induced peripheral antinociception in a dose-dependent manner. Additionally, the administration of the endocannabinoid metabolizing enzyme inhibitor (.5 μg per paw) or an AEA reuptake inhibitor, (5Z,8Z,11Z,14Z)N(4Hydroxy2methylphenyl)5,8,11,14 eicosatetraenamide (2.5 μg per paw) significantly enhanced low-dose ketamine-induced peripheral antinociception. AEA paw levels were increased only after ketamine administration to prostaglandin E_2-injected paws. These data suggest that ketamine, in the presence of a nociceptive stimulus, induces a selective release of AEA levels and subsequent CB_1 cannabinoid activation at the peripheral level.**

Perspective: ***This study suggests that ketamine antinociception depends at least in part on AEA release and CB_1 cannabinoid receptor activation in inflammatory conditions. This study could potentially help clinicians in the use of ketamine as a peripheral analgesic for inflammatory pain.***

Key words: *Ketamine, endocannabinoids, anandamide, cannabinoid receptors, peripheral antinociception.*

Ketamine was first tested clinically by Domino and co-authors in 1965[20] and, since then, has been extensively used for sedation, induction, and maintenance of general anesthesia, as well as premedication and postoperative analgesic.[59] In addition, there are reports of its use for the treatment of acute pain,[23] cancer,[45] neuropathic pain,[44] and burn,[65] as well as phantom limb pain.[3]

In addition to the classic mechanism of action of ketamine via the antagonism of the N-methyl-D-aspartate receptor, the modulation of other signaling pathways by ketamine has also been proposed, such as the inhibition

Received December 13, 2016; Revised August 11, 2017; Accepted December 3, 2017.

The research funding were provided by Fundação de Amparo a Pesquisa de Minas Gerais PPMFAPEMIG 2015 Process number 0474-15 and Fundação de Amparo a Pesquisa de Minas Gerais UNIVERSAL Process n° number 01307-14. Fellowship was provided by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

The authors have no conflicts of interest to declare.

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1526-5900/$36.00

https://doi.org/10.1016/j.jpain.2017.12.002

of voltage-gated calcium channels[66] and the activation of cholinergic,[1] serotonergic,[41] and opioidergic[5] systems. Furthermore, Romero and Duarte[54] showed the participation of the nitric oxide/cyclic guanosine monophosphate/adenosine triphosphate-sensitive potassium channel pathway in the peripheral antinociception that was induced by ketamine treatment.

However, the relationship between ketamine and the endocannabinoid system, consisting of 2 cannabinoid receptors (CBRs), type 1 (CB_1) and type 2 (CB_2), and of their endogenous ligands, anandamide (AEA) and 2-arachidonoylglycerol (2-AG) as well as enzymes for AEA and 2-AG biosynthesis and degradation, has not yet been elucidated. Data that could be used as a link between ketamine and the cannabinoid system were presented in the study by Hesselink and Kopsky.[32] These investigators reported that the combination of palmitoylethanolamide (PEA), an endocannabinoid-related molecule with no affinity for or direct activity at CBRs, and ketamine 10% cream, reduced pain in more than 50% of patients with chronic pain syndrome. This treatment also resulted in reductions in skin swelling. However, the relationship between PEA and the endogenous cannabinoid system is not clear; PEA has been classified as a CB_2 cannabinoid agonist[12,31,57] as well as a nuclear receptor peroxisome proliferator-activated receptor alpha agonist.[38]

CBRs CB_1 CB_2 were cloned in 1990 and 1993, respectively, as G protein-coupled receptors associated with $G_{i/0}$ subunits,[37] and activated by Cannabis psychotropic principle, Δ^9-tetrahydrocannabinol, as well as by the "endocannabinoids" AEA[17] and 2-AG.[42] The endocannabinoids are involved in several physiological and pathological functions, such as analgesia,[26] inflammation,[30] immunomodulation,[11] inhibition of tumor cell growth,[46] regulation of food intake,[7] and epilepsy.[39]

Several studies have shown the antinociceptive effect of CB_1 and CB_2 agonists[22] and ketamine.[19] However, the involvement of the endocannabinoid system in the antinociceptive effect of this latter drug has not been investigated to date. Therefore, the aim of this study was to determine whether or not activation of the endocannabinoid system could be triggered by the peripheral administration of ketamine.

Methods

Animals

All experiments used male Wistar rats weighing between 180 and 200 g (from CEBIO, Federal University of Minas Gerais, Minas Gerais, Brazil). The animals were placed in plastic boxes with forage shavings, with free access to water and food and kept in the trial room 2 days before the experiments, for habituation. They were housed in a temperature-controlled room (23°C ± 1°C), on an automatic 12-hour light/dark cycle. All tests were conducted during the light phase (8:00 AM to 5:00 PM). All animal procedures and protocols were approved by the Ethics Committee on Animal Experimentation of the Federal University of Minas Gerais and are in accordance with the recommendations for evaluation of experimental pain in animals.[67] After the experimental procedures, the animals were euthanized using an intraperitoneal injection of urethane 1.25 g/kg.

Measurement of Nociceptive Threshold

Hyperalgesia was induced using subcutaneous injection of prostaglandin E_2 (PGE_2; 2 μg) into the plantar surface of the hind paw. Hyperalgesia was measured using the mechanical paw pressure test described by Randall and Selitto.[49] An analgesiometer was used (Ugo-Basile SRL, Varese, Italy) with a cone-shaped paw-presser with a rounded tip, which applies a linearly increasing force to the hind paw. The weight in grams required to elicit the nociceptive response of paw withdrawal was determined as the nociceptive threshold. A cutoff value of 300 g was used to reduce the possibility of damage to the paws.

The nociceptive threshold was expressed in grams and it was determined in the right hind paw according to the average of 3 consecutive trials recorded before (0 time) and after PGE_2 injection (3 hours). The time course of responses was computed. The time of injections and time of nociceptive threshold measurements, as well as the doses used, were on the basis of literature data and on results of pilot experiments.[57]

Drug Administration

PGE_2 (2 μg; Enzo Life Sciences, Farmingdale, NY) was diluted in ethanol 2% whereas ketamine (10%; Vetbrands; Sao Paulo, Brazil), was dissolved in physiological saline. N(piperidine-1yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl 1 pyrazolcarboxamide, a CB_1 CBR antagonist (AM251; 20, 40, and 80 μg; Tocris, Pittsburgh, PA), 6-iodo-2-methyl-1-(2-morpholinoethyl)-1H-indol-3-yl) (4-methoxyphenyl) methanone, a CB_2 CBR antagonist (AM630; 100 μg; Tocris); (5Z,8Z, 11Z,14Z)-5,8,11,14-eicosatetraenyl-methylester phosphonofluoridic acid, an irreversible nonselective fatty acid amide hydrolase (FAAH) inhibitor, (MAFP; .5 μg; Tocris) and (5Z,8Z,11Z,14Z)N(4Hydroxy2methylphenyl)5,8,11,14 eicosatetraenamide, a selective inhibitor of AEA cellular reuptake, (VDM11, 2.5 μg, Tocris) were each diluted in 10% dimethyl sulfoxide in sterile saline. All of the aforementioned drugs were injected into the right plantar surface of the paw in a volume of 50 μL per paw, except PGE_2 (100 μL per paw).

Endocannabinoid Extraction and Quantification

Procedure of Extraction

The paw tissue from treated animals was extracted immediately after sacrifice. The tissue was homogenized in 2 vol of chloroform/methanol/Tris-HCl (50 mM; 2:1:1) containing 10 pmol of *d*8-AEA and *d*8-2-AG. Deuterated standards were synthesized from *d*8-arachidonic acid and ethanolamine or glycerol as described in Devane et al[17] and Bisogno et al,[9] respectively. Homogenates were