European Journal of Pharmacology 765 (2015) 68–74

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and analgesia

Synergistic antinociceptive interaction between palmitoylethanolamide and tramadol in the mouse formalin test

Myrna Déciga-Campos [a], Pamela Moncerrat Ramírez-Marín [a], Francisco Javier López-Muñoz [b,*]

[a] Sección de Estudios de Posgrado e Investigación, Escuela Superior de Medicina, Instituto Politécnico Nacional, México, D.F.11340, Mexico
[b] Departamento de Farmacobiología, Cinvestav-Sede Sur, México, D.F.14330, Mexico

ARTICLE INFO

Article history:
Received 18 February 2015
Received in revised form
7 August 2015
Accepted 17 August 2015
Available online 19 August 2015

Keywords:
Antinociception
N-palmitoylethanolamide
Tramadol
Isobolographic
Synergism

ABSTRACT

Pharmacological synergism has been used to obtain a higher efficacy using drug concentrations at which side effects are minimal. In this study, the pharmacological antinociceptive interaction between N-palmitoylethanolamide (PEA) and tramadol was investigated. The individual concentration–response curves for PEA (0.1–56.2 μg/paw) and tramadol (1–56.2 μg/paw) were evaluated in mice in which nociception was induced by an intraplantar injection of 2% formalin. Isobolographic analysis was used to evaluate the pharmacological interaction between PEA ($EC_{50}=23.7\pm1.6$ μg/paw) and tramadol ($EC_{50}=26.02\pm2.96$ μg/paw) using the EC_{50} and a fixed 1:1 ratio combination. The isobologram demonstrated that the combinations investigated in this study produced a synergistic interaction; the experimental values ($Z_{exp}=9.5\pm0.2$ μg/paw) were significantly smaller than those calculated theoretically ($Z_{add}=24.8\pm0.2$ μg/paw). The antinociceptive mechanisms of the PEA and tramadol combination involved the opioid receptor, transient receptor potential cation channel subfamily V member 1 (TRPV1), and peroxisome proliferator-activated receptor alpha (PPAR-α). The sedative effect of the combination of PEA and tramadol was less than that generated by individual treatments. These findings suggest that the PEA and tramadol combination produced enhanced antinociceptive efficacy at concentrations at which side effects are minimal.

1. Introduction

It has been suggested that a combination of antinociceptive drugs with different mechanisms of action could be more efficacious by working synergistically. This strategy could achieve pharmacological effects at lower concentrations of each agent and therefore reduce the intensity and incidence of unwanted effects.

It is widely known that activation of either opioid or cannabinoid systems produce antinociceptive properties in various pain models. Many studies have shown this synergistic antinociceptive interaction between cannabinoid and opioid systems (Manzanares et al., 1998, 1999; Cichewicz 2004; Cox et al., 2007; Seely et al., 2012). However, the use of cannabis is frequently underreported in our society. Despite legal restrictions, cannabis is often used to relieve chronic and neuropathic pain (Mao et al., 2000). Unfortunately, this agent is associated with adverse psychotropic and physical effects and possesses a potential for addiction. Morphine also produces many adverse effects including analgesic tolerance, nausea, sedation, and constipation. The involvement of these endogenous systems in pain control suggests an interesting alternative that would take advantage of this pharmacological mechanism. Many studies have demonstrated that the endogenous opioid system could be involved in cannabinoid antinociception, and there is also evidence for a role of the endogenous cannabinoid system in opioid antinociception (Cichewicz, 2004). These interactions may lead to additive or even synergistic antinociceptive effects, emphasising their clinical relevance in human to enhance analgesic effects at lower concentrations with consequently fewer undesirable side effects.

N-palmitoylethanolamide (PEA) is an endogenous fatty acid amide and is a structural analogue of anandamide (arachidonoyl ethanolamide, AEA). PEA has been isolated from soy lecithin, soybean, peanut meal and egg yolks (Kuehl et al., 1957) and is abundant in plants, particularly in seeds (Kim et al., 2013). Synthesis and degradation of PEA occur in various cell types, including those relevant for chronic pain and inflammation signalling, such as immune cells, neurons and microglia (Skaper and Facci, 2012). Several reports note that PEA has antinociceptive,

* Correspondence to: Lab. No. 7 "Dolor y Analgesia" del Departamento de Farmacobiología, Cinvestav-Sede Sur, Calz. de los Tenorios No. 235 Col. Granjas Coapa, Deleg. Tlálpan, México D.F., C.P. 14330, Mexico. Fax: +52 55 54832863.
E-mail addresses: flopez@cinvestav.mx, flopezm@prodigy.net.mx (F.J. López-Muñoz).

http://dx.doi.org/10.1016/j.ejphar.2015.08.025
0014-2999/

anti-hyperalgesic and anti-allodynic properties (Costa et al., 2008; Romero and Duarte 2012). The proposed mechanisms of action include the "entourage effect:" anandamide potentiation effect through the cannabinoid or transient receptor potential cation channel subfamily V member 1 or "TRPV1" (De Petrocellis et al., 2001; Smart et al., 2002). The peroxisome proliferator-activated receptor alpha (PPAR-α) is also involved in the antinociceptive and anti-inflammatory effects of PEA (Fehrenbacher et al., 2009; D'Agostino et al., 2009). Tramadol, a synthetic opioid, is generally well tolerated and causes few adverse opioid-type effects. This drug is commonly used to treat acute and chronic pain. Tramadol exhibits both mild opioid receptor-binding and norepinephrine and serotonin reuptake inhibition (Driessen and Reimann, 1992; Driessen et al., 1993). Both these opioid and non-opioid mechanisms contribute independently to the antinociceptive effect of tramadol. The present work evaluated the synergistic antinociceptive interaction of combinations of low concentrations of an endocannabinoid, PEA, and a weak opioid, tramadol; in addition, it analysed some of the drugs' mechanisms of action and adverse sedative effect.

2. Materials and methods

2.1. Animals

Female *Swiss Webster* mice (body weight range, 25–30 g) from the Pharmacobiology Department in the Centre of Research and Advanced Studies were used in this study. The animals were housed under standard light/dark cycle (lights on 7:00 a.m.) and constant temperature (22–24 °C) conditions and had free access to food and drinking water before the experiments. All experiments were performed between 7:30 a.m. and 3:00 p.m. All experimental procedures followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (IASP, 1983; Zimmermann, 1983). The experimental protocol was approved by the local Institutional Animal Care and Use Committee in accordance with the Mexican federal regulations for the Care and Use of Laboratory Animals NOM-062-ZOO-1999 (Mexican Ministry of Health). Each mouse was used only once during the protocol and was sacrificed in a CO_2 chamber immediately after the experiment. For all experimental procedures, the groups were composed of at least six mice.

2.2. Drugs

A solution of formaldehyde (37%) (PubChem CID: 712) was purchased from J.T. Baker (Pennsylvania, USA); PEA (PubChem CID: 4671), naloxone hydrochloride (PubChem CID: 5464092, capsazepine (PubChem CID: 4671), and GW6471 (PubChem CID: 71311842) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Tramadol (PubChem CID 10017651) was provided by Grünenthal S.A de C.V, Mexico. PEA was suspended in the vehicle (20% dimethyl sulfoxide in saline solution (0.9% w/v)). Naloxone, capsazepine, and GW6471 were prepared in a saline solution (0.9% w/v). All substances were freshly prepared for each use and administered subcutaneously (s.c.) into the right hind paw of the mouse with a 30-gauge needle. The formaldehyde was dissolved in distilled water. The control animals received the same volume of the vehicle (20% dimethyl sulfoxide in saline solution (0.9% w/v)).

2.3. Pharmacological evaluation

2.3.1. Study design

A range of concentrations of PEA (0.1, 10, 31.6, 56.2 μg/paw), tramadol (1, 10, 31.6, 56.2 μg/paw) or the PEA+tramadol combination (see Table 1) were administered to groups of at least six mice for each condition. All drugs were administered 15 min before the evaluation of spontaneous locomotor activity, and then each mouse was subjected to the formalin test. The time schedule for drug administration was determined in a pilot experiment in our laboratory (data not shown).

Table 1
Concentration used in this study the interaction between PEA and tramadol in the formalin test in mice.

Concentration in combination (μg/paw), 1:1 ratio		
PEA	Tramadol	Total concentration
4.3	4.5	8.8
8.6	9.1	17.7
17.3	18.2	35.5
34.6	36.5	71.1
69.2	73	142.2

2.3.2. Spontaneous locomotor activity

The sedative activity of PEA, tramadol and their combination was determined in mice as the effects on spontaneous locomotor activity. Fifty min after the injection of PEA, tramadol or the combination, the mice were placed one at a time in the centre of an acrylic cage that was divided into 12 squares (4 cmx5 cm). The number of squares explored and the rearing up demonstrated by each mouse in a 2 min interval was recorded as the ambulatory activity (Montiel-Ruiz et al., 2012). After this evaluation, the mice were subjected to the formalin test.

2.3.3. Formalin test

This experimental model consisted of placing each mouse in an open Plexiglas observation chamber with mirrors for 30 min to allow them to become accustomed to their surroundings. Each mouse was then removed for formalin administration; 20 μl of diluted 2% formalin was administered subcutaneously (s.c.) into the right hind paw of the mouse with a 30-gauge needle. The amount of time of spent licking the injected paw was defined as a nociceptive response, which was recorded in 5-min intervals during a 30-min period after the algesic injection (Hunskaar and Hole, 1987). The formalin-induced licking behaviour was biphasic: the initial acute phase (first phase, 0–10 min) was followed by a relatively short quiescent period that was then followed by a prolonged tonic response (second phase, 15–30 min) (Hunskaar and Hole, 1987; Shibata et al., 1989).

2.4. Antinociceptive mechanism of action of the PEA–tramadol combination

PEA (34.6 μg/paw), tramadol (36.5 μg/paw) and their combination in a 1:1 ratio (71.1 μg/paw) were used to investigate the possible mechanism of action. Before the administration of PEA, tramadol or their combination, the animals were given 20 μg/paw naloxone (an opioid receptor antagonist) or 10 μg/paw GW6471 (a PPAR-α antagonist). Fifteen min later, the PEA, tramadol, the combination of the two drugs or vehicle were administered, and then (after an additional 15 min) each animal received an intraplantar injection of 20 μL of 2% formalin to induce the nociceptive behaviour as described above. To evaluate the role of TRPV1, 300 μg/paw capsazepine (a TRPV1 antagonist) was injected 2 h before the administration of PEA, tramadol or their combination; formalin was injected and nociceptive behaviour was evaluated. The time schedule for capsazepine injection was determined in a pilot experiment in our laboratory (data not shown).