Journal of Behavioral and Brain Science, 2022, 12, 627-639
https://www.scirp.org/journal/jbbs
ISSN Online: 2160-5874
ISSN Print: 2160-5866

PGE2 as a Morphine-Seeking Behavior Modulator in the Place Preference Model

Ariadna Jiménez-González[1,2], Abraham Ochoa-Aguilar[2], Katia Mendoza-Negrete[1], Leticia Parra-Gamez[3], Claudia Gómez-Acevedo[1*]

[1]Department of Pharmacology, Behavioral Pharmacology Laboratory, Facultad de Medicina, Universidad Nacional Autónoma de México (UNAM), México City, México
[2]Hospital Loma Linda, Translational Research Laboratory, Naucalpan, México
[3]Department of Anatomy, Facultad de Medicina, Universidad Nacional Autónoma de México (UNAM), México City, México
Email: *goac@unam.mx

How to cite this paper: Jiménez-González, A., Ochoa-Aguilar, A., Mendoza-Negrete, K., Parra-Gamez, L. and Gómez-Acevedo, C. (2022) PGE2 as a Morphine-Seeking Behavior Modulator in the Place Preference Model. *Journal of Behavioral and Brain Science*, **12**, 627-639.
https://doi.org/10.4236/jbbs.2022.1212037

Received: October 18, 2022
Accepted: December 5, 2022
Published: December 8, 2022

Abstract

The opioid epidemic has become one of the most concerning public health issues in the world, and currently does not have an adequate treatment available. It has been observed that, despite opioids being highly addictive, patients with chronic inflammation are less likely to develop an opioid dependence. This protective effect may be caused by Prostaglandin E2 (PGE2) as it has been found that non-painful carrageenan inflammation reduces morphine induced reward. Taking this into account, the aim of this study was to determine if the direct administration of PGE2 into the central nervous system could modulate the morphine-induced reward. We used the morphine-conditioned place preference (CPP) model with and without PGE2 or PGE2R antagonist in order to test the reward response. We found a significant reduction of morphine-induced reward after administering PGE2. Moreover, we found that this effect could be reversed by PGE2 receptor antagonism. Our data suggest that PGE2 may reduce morphine-induced reward making it an important drug-target research alternative to explore the possibility of modifying or even preventing opioid addiction.

Keywords

Morphine Addiction, Conditioned Place Preference, Rats, Prostaglandins, Immune System, Reward

1. Introduction

Opioid addiction is a chronic and relapsing disorder regarded as one of the most severe public health concerns [1]. According to WHO, about 70% of the deaths

attributed to drugs worldwide are related to opioids [2]. Additionally, opioid dependence leads to infections, social disintegration, violence, and crime [3]. However, opioids are a broad group of pain relievers consider as some of the most effective analgesic drugs for acute and chronic pain treatment [4]. Morphine is the most representative and studied of all opioids, so it's of great importance to counteract its adverse effects, such as the development of addiction.

The pathophysiology of a drug addictive process is very complex since it involves the function alteration of the motivation and reward system, originally described by Olds and Milner [5]. One of the main mechanisms for the development of opioid addiction begins with the release of dopamine (DA) from the ventral tegmental area (VTA), which is part of the reward system [6]. This release is mainly due to the activation of Mu opioid receptors which are a Gi-type of metabotropic receptors. This activation causes inhibition of GABAergic interneurons that project onto dopaminergic neurons in the VTA [7] [8]. Once released, DA binds to D1and D2 receptors in the Nucleous Accumbens (NAc). Such interactions have shown to play a fundamental role in the reward and motivation for opioid use [9].

For a long time, the aforementioned process was considered merely a neuronal event, but recent studies have found that the immune system (IS) plays a predominant role in opioid addiction [10]. Clinical studies have shown that there is a lower opioid addiction incidence in patients with chronic pain (0.19%), compared to the general population (10%) [11]. In addition to this, some animal models have shown that peripheral inflammation can affect DA release in the central nervous system (CNS) [12], and that carrageenan-induced painful inflammation decreases morphine reward in the conditioning place preference model and the morphine withdrawal syndrome [13] [14] [15]. This evidence suggests that a low susceptibility to developing an opioid dependence could be attributed to a pain process. However, we recently found that carrageenan-induced painless inflammation is responsible for blocking morphine reward behavior. Moreover, the administration of ibuprofen significantly increases reward behavior and at the same time blocks the inflammation processes [13]. We speculate that the cause for this is related to the increase of pro-inflammatory molecules which is induced by carrageenan administration and modulated by administering ibuprofen. Particularly, it has been observed that inflammation caused by intraplantar administration of carrageenan is closely related to a local, systemic, and CNS elevation of PGE2 [16]. It was also observed that the administration of ibuprofen inhibits the necessary enzyme for prostaglandin synthesis [17]. On the other hand, PGE2 has also shown to have a neuroprotective [18] and anti-inflammatory effect [19] [20] [21], which appears to be actively involved in impulsivity, social aversion, and stress-induced depression [22] [23].

Based on the previously described studies, we hypothesized that the mechanism by which peripheral inflammation has generated a modulation in reward processes and withdrawal syndrome is strongly related to prostaglandins. How-

ever, it has not been determined yet whether there exist a relationship between PGE2 concentration in the CNS and the process of opioid induced addiction. Therefore, the aim of this study was to evaluate whether the administration of PGE2 in the CNS causes modifications in reward behaviors induced by morphine. Finally, the PGE2 pharmacological characterization was carried out in order to determine if there is a fundamental molecule in the PGE2 metabolism or a specific receptor necessary for it to exert an impact in the addictive process.

2. Materials and Methods

Subjects

Adult male Wistar rats (n = 70) weighing between 250 - 350 g at the start of experiments were used for this study. Rats were housed in a temperature- and humidity-controlled vivarium with *ad libitum* access to water and food in an inverted light/dark cycle (*i.e.* lights switching on at 7 pm and switching off at 7 am). Rats were first subjected to surgery and then housed in groups of five for 7 days up to the start of the behavioral experiments. Experiments were conducted in a room with dim red lights during the dark part of the cycle and external noise cancellation. All experiments were performed in accordance with the National Institute of Health Guide for the care and use of laboratory animals and were approved by the Ethics Committee of the Universidad Nacional Autónoma de México (UNAM; FM/DI/074/2017).

Intracranial cannulation surgery

The intracranial cannulation surgery has been described previously [24]. On the first day, rats were anesthetized with xylazine (10 mg/kg) and ketamine (75 mg/kg) and placed in the stereotaxic instrument. A bilateral guide cannula of 24 G was implanted in both lateral ventriculus, then they were anchored to the skull with two stainless steel screws and polycarboxylate cement. The stereotaxic coordinates used were DV: 3.5 mm, AP: 0.8 mm, and ML: 1.2 mm obtained from the Paxinos rat brain atlas [25]. All animals were allowed to recover for at least 7 days before the place preference conditioning procedure started.

Drugs

Morphine sulphate (BioGenTec®) was diluted in sterile saline solution (NaCl 0.9%) and 5 mg/kg were administered subcutaneously (*s.c*). 5μg of PGE2 (Sigma-Aldrich®) were diluted in 5 μL of sterile pyrogen-free distilled water and administered over 2 minutes in both ventriculus (*i.c.v*). AH6809 (Sigma-Aldrich®), the EP2 antagonist, was dissolved in sterile saline solution (NaCl 0.9%) and administered intraperitoneally (*i.p.*) at 500 μg/kg.

Conditioned place preference apparatus

Conditioning sessions were performed in a three-chamber apparatus (65 cm wide × 30 cm deep × 30 cm high). Lateral compartments (25 × 30 × 30 cm each) had different physical conditions: Compartment A had white walls and mash floor while compartment B had black stripe walls and smooth floor. The central (neutral) compartment (10 × 30 × 30 cm) had black walls and smooth floor

(Compartment C) and was connected to both compartments through removable doors. Also, each compartment had a different amount of led white light: 100 luxes in the A compartment; 0.8 luxes in the B Compartment; and 18 luxes for the C compartment. All experiments were recorded with a video camera and analyzed with a movement analyzer program (Omnialva®). After every session rat urine and feces were removed and the apparatus was cleaned with a 70% ethanol solution.

Place preference conditioning procedure

The conditioning place preference test was performed 7 days after surgery. As Tomazi reported [26] the test had a six day duration and it was divided in three phases:

Pre-conditioning test phase

On day one, rats were placed in the middle compartment; all doors opened allowing them to freely explore the compartments. All rats were recorded for 900 seconds to determine the basal compartment preference. Since we used a biased model [27], rats that failed to show preference for the B compartment were eliminated from subsequent CPP experiments.

Conditioning test phase

Conditioning sessions were performed for 4 days during which saline and morphine were alternately administered to rats. Rats received a subcutaneous saline administration at 8:00 h; then they were placed at B compartment for 30 min and returned to their home cage. 6 hours later, the second conditioning session started with a 5 mg/kg morphine subcutaneous administration. Immediately after the rat was placed at A compartment for 30 min. The next day, the conditioning session started by administering morphine at 8:00 h, as shown in **Figure 1**. 10 minutes before that, an internal cannula of 1mm projection beyond the tip of the guide cannula was connected. 5μg of PGE2 diluted in 5 μL of sterile, pyrogen-free, distilled water or vehicle solution were administered over 2 minutes in both ventriculus. Immediately before 500 μg/kg PGE2 AH6809 an EP2 antagonist that has the highest affinity for the EP2 receptor [28] were administered intraperitoneally. AH6809 is an EP2 antagonist and has the highest affinity for the EP2 receptor [28].

Post-conditioning test phase

On the last day of the experiment, rats were allowed to freely explore the apparatus for 900 seconds and conditioned preference was recorded and analyzed.

3. Experimental Design

Experiment* 1: *to determine if mere manipulation or the administration of vehicle or PGE2 could elicit reward.

In this experiment, animals were surgically prepared with a guide cannula as it was previously described. Then, conditioned place preference was performed in 4 separated groups which were design to elucidate if surgical manipulation or the administration of saline, PGE2 vehicle or PGE2 might trigger reward: 1) Sa-

line control (S) received sterile saline solution (*s.c*); 2) Saline sham (SS) received sterile saline solution (*s.c.*) after intracranial cannulation surgery manipulation; 3) Saline vehicle (SV) received sterile saline solution (*s.c.*) and 5 µl of PGE2 vehicle (*i.c.v*). 4) Saline PGE2 (SP) received sterile saline solution (*s.c.*) and PGE2 5 µg in 5 µl (*i.c.v.*).

Experiment* 2: *to determine if administering morphine induced reward

In this experiment, animals received morphine (5 mg/kg, *s.c.*) after being surgically prepared with a guide cannula as mentioned before. Then, conditioned place preference was performed in 3 separate groups which were designed to identify if surgical manipulation or if the administration of saline or morphine vehicle could modify morphine induced reward: 1) Morphine control (M) received 5 mg/kg of morphine (*s.c.*); 2) Sham morphine (SM) received 5 mg/kg of morphine (s.c.) after intracranial cannulation surgery manipulation; 3) Morphine vehicle (MV) received morphine 5 mg/kg (*s.c.*) and 5 µl of morphine vehicle (*i.c.v.*).

Experiment* 3: *to determine if PGE2 administration modifies morphine-induced reward

In this experiment, animals received morphine (5 mg/kg, *s.c.*) after being surgically prepared with a guide cannula as previously described. Then conditioned place preference was performed in 2 separated groups which were designed to identify if the administration of PGE2 could modify morphine induced reward: 1) Morphine vehicle (MV) received morphine 5 mg/kg (*s.c.*) and 5 µl of morphine vehicle (*i.c.v.*); 2) Morphine PGE2 (MP) received morphine 5 mg/kg (*s.c.*) and PEG2 (5 µg in 5 µl, *i.c.v*).

Experiment* 4: *to determine if EP2 antagonist administration could modify the effect of PGE2 in morphine-induced reward

In this experiment, animals received morphine after being surgically prepared with the guide cannula as described above. Then conditioned place preference was performed in a separated group which was designed to identify if EP2 antagonist AH6809 administration might block the effect after PGE2 administration: Morphine PGE2 antagonist (MPA) animals received morphine (5 mg/kg, *s.c.*), PEG2 (5 µg in 5 µl, *i.c.v*) and AH6809 (500 µg/kg, *i.p.*).

Cannula placement verification and histology

At the end of experiments, rats were euthanized with a pentobarbital overdose. After decapitation, their brains were removed and fixed in hematoxylin/eosin. Coronal sections (40 µm thick) of brain tissue were sliced on a cryostat to verify the infusion sites (Figure 1).

Data Analysis

Behavioral results were integrated in the CPP score calculated by subtracting the total time (seconds) spent in the non-preferred compartment before conditioning to the total time (seconds) spent in the non-preferred compartment after conditioning.

All data were analyzed using Prism® 9 software. The results are present as a means group ± SEM (Standard Error of Mean). The normality of data distribution

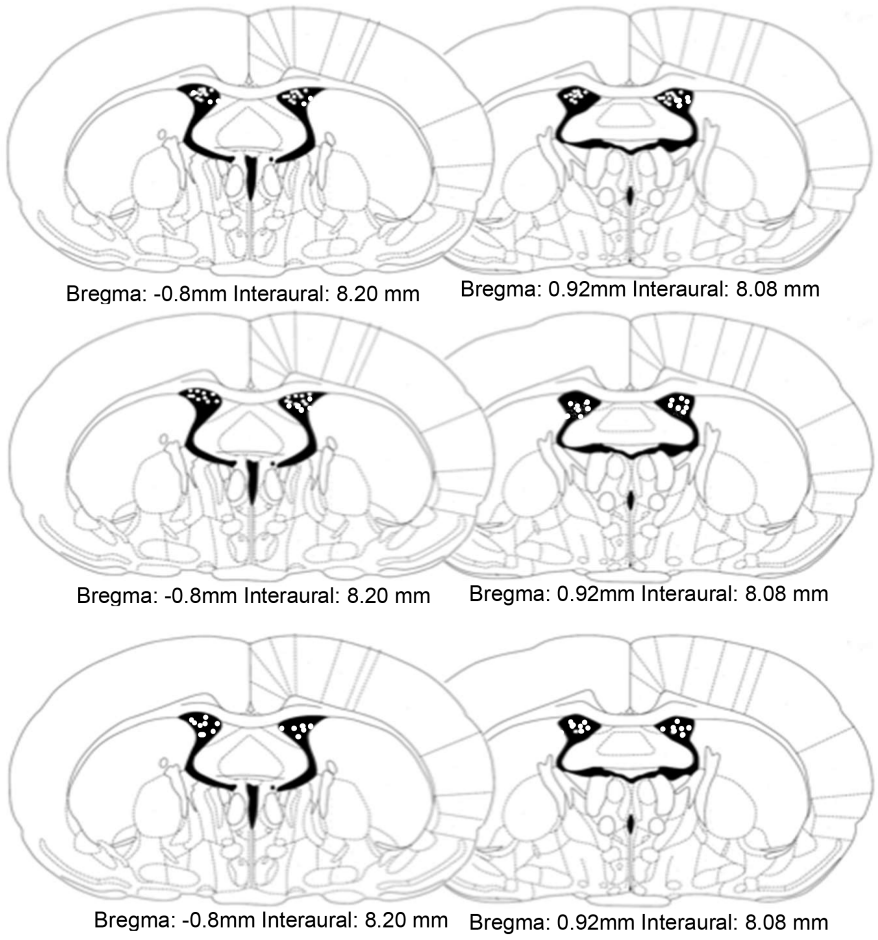

Figure 1. Diagrams of coronal rat brain sections (Paxinos and Watson, 1998). The white dots represent the site of cannula insertion in the ventricular area.

was calculated using the D'Agostino & Pearson omnibus normality test. Statistical differences between groups were evaluated through one way analysis of variance (ANOVA) followed by post hoc comparisons using Tukey test if statistically significant effect were observed in the ANOVAs. Differences were considered as significant at $P < 0.5$.

4. Results

***Experiment* 1**

The CCP scores obtained showed that the administration of saline solution failed to induce place preference (CCP score of −143.5, n = 10 rats). Similarly, after the surgical manipulation and PGE2 vehicle or PGE2 (*i.c.v*) administration rats chose the saline-paired compartment (CCP scores of −92.38 and −55.93, respectively; n = 10 rats in each group). Therefore, ordinary one-way ANOVA showed non-significant difference between these groups ($F_{(3,36)} = 2.773$; $P = 0.0554$; Figure 2).

Experiment* 2: *Morphine-induced reward

Our results revealed that all groups treated with morphine showed induced

place preference (Figure 3). The data obtained in this study showed that 5 mg/kg (*s.c.*) of morphine induced an important reward (CCP score of 236.4, n = 10 rats). The effects of morphine effect were not modified by surgery manipulation nor by the *i.c.v.* administration of vehicle (CCP score of 354 and 331.5, respectively, n = 10 rats in each group). Ordinary one-way ANOVA showed non-significant difference between these 3 groups ($F_{(2,27)} = 2.817$; P = 0.0774). Additionally, the comparison between groups that received (*s.c.*) morphine (5 mg/kg, MV) or saline solution (SV) and then a vehicle (*i.c.v.*) showed a significant difference in the place preference induced by morphine administration (CCP scores of 331.5 vs. −35.36, n = 10 rats, P < 0.01, ($F_{(2,27)} = 36.21$; P < 0.0001).

***Experiment* 3: *PGE2 blocks place preference induced by mo*rphine**

As Figure 4 shows, the administration of 5 μ of PGE2 significantly ($F_{(2,27)} = 19.64$, P < 0.001) reduced the time spent at the morphine paired compartment,

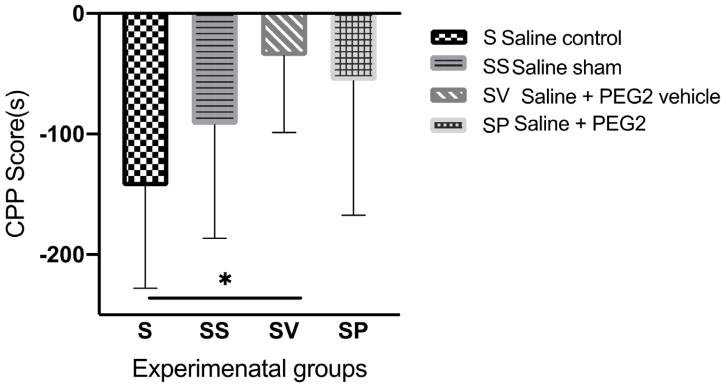

Figure 2. Effect of different control conditions in the CPP paradigm. Administration of saline solution failed to induced place preference, as shown by the CPP score. Additionally, after surgical manipulation and administration of PGE2 or PGE2 vehicle, rats chose the saline-paired compartment. Ordinary one-way ANOVA showed no significant difference between these groups ($F_{(3,36)} = 2.773$; P = 0.0554, n = 10 rats each group, *Tukey post-hoc P = 0.0509).

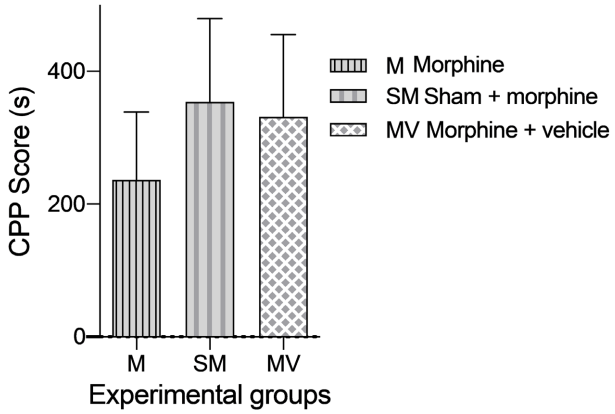

Figure 3. The administration of morphine (5 mg/kg) induced important reward. The effect of morphine was not modified by surgery manipulation nor the *i.c.v.* administration of vehicle. Ordinary one-way ANOVA showed non-significant difference between these 3 groups ($F_{(2,27)} = 2.817$; P = 0.0774).

in comparison to the administration of vehicle. The CCP score of the group of animals (n = 10) with morphine was 331.50, while those animals that received prostaglandin E2 (*i.c.v.*) after the morphine induced place preference scored −63.57.

Experiment* 4: *EP2 receptor antagonist effect

Administration of EP2 receptor antagonist (AH6809) reversed the effect induced by PGE2 in the morphine induced place preference as Figure 5 illustrates. Our data showed that 500 μg/kg of EP2 receptor antagonist (AH6809) intraperitoneally administered was able to block the effect induced by the administration of PGE2 (CCP scores 180.3 and −63.57, n = 10 each group, $F_{(2,27)} = 19.64$, $P < 0.001$). No significant difference was found between the MV group and the MPA group.

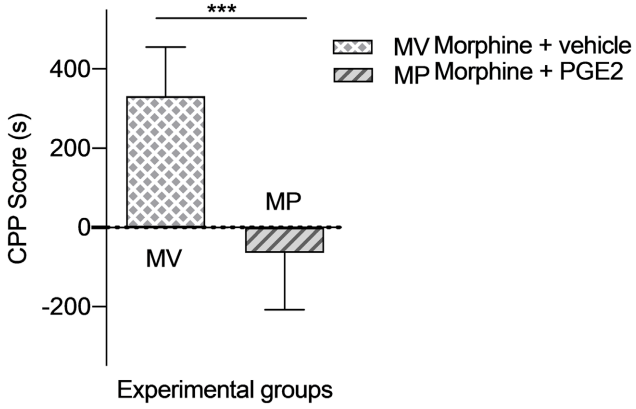

Figure 4. Administration of PGE2 significantly reduced the time spent at the morphine paired compartment, as shown by the CPP scores. The group of animals with morphine (n = 10) CCP score was 331.50, while the animals that received prostaglandin E2 (*i.c.v.*) after the morphine induced place preference score −63.57 ($F_{(2,27)} = 19.64$, $P < 0.001$).

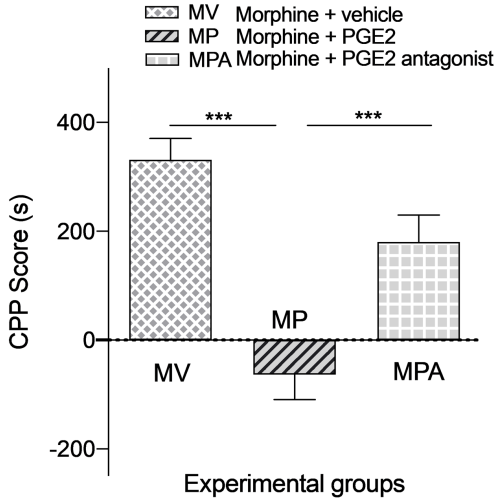

Figure 5. Administration of EP2 receptor antagonist (AH6809, 500 μg/kg) significantly reversed the effect induced with PGE2 in the morphine induced place preference paradigm, as shown by the CPP scores. (CCP scores 331.5, −63.57, 180.3, n = 10 each group, $F_{(2,27)} = 19.64$, ***Tukey post-hoc, $P < 0.001$).

5. Discussion

The most important finding of this study is that administration of PGE2 provoked a significant decrease in the morphine-induced place preference. Furthermore, the effect in the reward response was blocked with an EP2 receptor antagonist administration. This influence in the reward behavior induced by PGE2 raises the possibility that the eicosanoid could have opioid addiction protection qualities.

As mentioned before, the immune system is an active part of the opioid addiction process. Clinical evidence has shown that chronic pain patients exposed to opioid treatment would develop opioid addiction in a smaller percentage. Only about 0.19% of the patients showed a history of opioid abuse compared to the 10% of the general population [11]. Such disparity raises the question of whether inflammation provides a protective effect. In fact, the evidence that inflammation is related to addictive processes has been demonstrated thoroughly in recent years. For example, it has been shown that morphine can activate microglia through the activation of TLR4 receptors, in a similar way to that induced by the endotoxin liposaccharide, consequently conducting chronic neuroinflammation [29]. Also, it has been found that when opioids are systemically and acutely administered they elicit a TLR dependent response in the Central Nervous System (CNS), but a chronic administration generates an IL-1b and TNF-α elevation [29]. Also, it has been suggested that the cytokines secretion may contribute to a pro-algesic effect, that increases glutamatergic and dopaminergic concentrations, which in turn increases opioid dependence and addictive effects. However, this effect may be detrimental to the immune system response since there is evidence showing that exposure of human natural killer cells to morphine decreases their ability to induce apoptosis. This response was prevented using a TLR4 selective inhibitor [30].

Recent findings, ours included, have shown that painful and non-painful inflammation downregulates opioid reward-induced behavior. Suzuki reported in 1996 that painful inflammation induced with the administration of carrageenan attenuated morphine-conditioned place preference [15]. Then it was shown that administration of ibudilast, an anti-inflammatory phosphodiesterase inhibitor and glial regulator, reduced extracellular dopamine concentration in Nucleus Accumbens [31]. This reduction significantly inhibited the opioid-induced reward [32]. Later, we reported the non-painful carrageenan induced inflammation had protective effects to morphine-induced conditioned place preference [13]. Additionally, we found this effect was reversed by administering a cyclooxygenase inhibitor. These results showed that the protective effect of non-painful inflammation was related to PGE2 concentration since the administration of a synthetic prostaglandin metabolic route inhibitor led to a reestablishment of morphine induced reward behaviors. Furthermore, the results presented here confirm the previously suggested protective effect for PGE2: the coadmini-

stration of an antagonist of the PGE2 receptor caused a significant reduction of the PGE2 protective effect (**Figure 5**). However, we only tested an acute dose of the PGE2 antagonist, therefore it will be important to evaluate in the future if the protective effect of the drug presented here shows similar conditions when administered at different doses.

Prostaglandins are membrane derived lipid eicosanoids with a wide variety of immune and non-immune functions. PGE2 quickly and widely suppresses TLR4 via intracellular cAMP increment [33]. In fact, it has been found that PGE2 administration decreases 40% the expression of TLR4 in macrophages which, in turn, decreases TNFα liberation [21]. The results of this study suggest that PGE2 administration decrease the expression of the TLR4 receptor in microglia cells. Said decrease is cAMP-dependent in the Central Nervous System, in a similar way as Degraff *et al.* (2014) observed in alveolar macrophages. Therefore, this low concentration of TLR4 receptors could lead to a lower morphine-induced neuroinflammation reaction as Wang *et al.* (2012) described. This leads to a lower probability of inducing its reinforcing and addictive effects.

6. Conclusion

Opioid addiction is a chronic disease that has led to a severe health crisis. Last year, the United States (US) reported 136 daily deaths caused by opioid overdose. An epidemiological analysis found that 0.4% of the population in the U.S. between the ages of 15 and 64 uses opioids, and up to 23% of them will become addicted in the following years [34]. Many steps are being taken in order to stop this situation, but the lack of treatment available for opioid addicts is detrimental to those efforts. Searching for alternative treatment targets is fundamental; in this sense, experts of the National Institute on Drug Abuse recognize that other potential directions in the search and development of new medications could include epigenetic, microRNA and neuroimmune targets [1]. The results presented in this study show, for the first time, that administration of PGE2 has a protective effect on morphine induced addictive behaviour. Although the results may not yet be relevant as an option for clinical treatment, they represent a new paradigm to drug addiction and its potential treatment.

Acknowledgements

This study was supported by Facultad de Medicina, Universidad Nacional Autónoma de México (UNAM), and PAPIIT (IN207918). Ariadna Jiménez-González is a doctoral student from Programa de Doctorado en Ciencias Biomédicas, UNAM; and received the fellowship 889179 from CONACYT. All experiments were approved by the ethical committee of Facultad de Medicina (UNAM; FM/DI/074/2017). Authors want to thank Miss Brenda Sandoval Meza, Head of the Scientific Paper Translation Department, from División de Investigación at Facultad de Medicina, UNAM, for editing the English-language version of this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Volkow, N.D. and Blanco, C. (2021) The Changing Opioid Crisis: Development, Challenges and Opportunities. *Molecular Psychiatry*, **26**, 218-233. https://doi.org/10.1038/s41380-020-0661-4

[2] WHO (2021) WHO Opioid Overdose. https://www.who.int/news-room/fact-sheets/detail/opioid-overdose

[3] Oviedo-Joekes, E., *et al.* (2009) Diacetylmorphine versus Methadone for the Treatment of Opioid Addiction. *New England Journal of Medicine*, **361**, 777-786. https://doi.org/10.1056/NEJMoa0810635

[4] Volkow, N.D., Jones, E.B., Einstein, E.B. and Wargo, E.M. (2019) Prevention and Treatment of Opioid Misuse and Addiction: A Review. *JAMA Psychiatry*, **76**, 208-216. https://doi.org/10.1001/jamapsychiatry.2018.3126

[5] Olds, J. and Milner, P. (1954) Positive Reinforcement Produced by Electrical Stimulation of Septal Area and Other Regions of Rat Brain. *Journal of Comparative and Physiological Psychology*, **47**, 419-427. https://doi.org/10.1037/h0058775

[6] Koob, G.F. and Volkow, N.D. (2016) Neurobiology of Addiction: A Neurocircuitry Analysis. *The Lancet Psychiatry*, **3**, 760-773. https://doi.org/10.1016/S2215-0366(16)00104-8

[7] MacNicol, B. (2017) The Biology of Addiction. *Canadian Journal of Anesthesia/Journal Canadien d'Anesthésie*, **64**, 141-148. https://doi.org/10.1007/s12630-016-0771-2

[8] Matsui, A. and Williams, J.T. (2011) Opioid-Sensitive GABA Inputs from Rostromedial Tegmental Nucleus Synapse onto Midbrain Dopamine Neurons. *Journal of Neuroscience*, **31**, 17729-17735. https://doi.org/10.1523/JNEUROSCI.4570-11.2011

[9] Steinberg, E.E., Boivin, J.R., Saunders, B.T., Witten, I.B., Deisseroth, K. and Janak, P.H. (2014) Positive Reinforcement Mediated by Midbrain Dopamine Neurons Requires D1 and D2 Receptor Activation in the Nucleus Accumbens. *PLOS ONE*, **9**, e94771. https://doi.org/10.1371/journal.pone.0094771

[10] Cui, C., Shurtleff, D. and Harris, R.A. (2014) Neuroimmune Mechanisms of Alcohol and Drug Addiction. In: *International Review of Neurobiology*, Elsevier, Amsterdam, 1-12. https://doi.org/10.1016/B978-0-12-801284-0.00001-4

[11] Fishbain, D.A., *et al.* (2007) What Percentage of Chronic Nonmalignant Pain Patients Exposed to Chronic Opioid Analgesic Therapy Develop Abuse/Addiction and/or Aberrant Drug-Related Behaviors? A Structured Evidence-Based Review. *Pain Medicine*, **9**, 444-459. https://doi.org/10.1111/j.1526-4637.2007.00370.x

[12] Felger, J.C. and Miller, A.H. (2012) Cytokine Effects on the Basal Ganglia and Dopamine Function: The Subcortical Source of Inflammatory Malaise. *Frontiers in Neuroendocrinology*, **33**, 315-327. https://doi.org/10.1016/j.yfrne.2012.09.003

[13] Sotomayor-Sobrino, M.A., *et al.* (2018) Non-Painful Peripheral Inflammation Blocks Conditioned Place-Preference to Morphine and Nicotine through an Ibuprofen-Sensitive and an Ibuprofen Insensitive Pathway. *Journal of Behavioral and Brain Science*, **8**, 57-76. https://doi.org/10.4236/jbbs.2018.82004

[14] Vaccarino, A.L., *et al.* (1993) Morphine Fails to Produce Tolerance When Administered in the Presence of Formalin Pain in Rats. *Brain Research*, **627**, 287-290. https://doi.org/10.1016/0006-8993(93)90332-H

[15] Suzuki, T., Kishimoto, Y. and Misawa, M. (1996) Formalin- and Carrageenan-Induced Inflammation Attenuates Place Preferences Produced by Morphine, Methamphetamine and Cocaine. *Life Sciences*, **59**, 1667-1674. https://doi.org/10.1016/0024-3205(96)00498-5

[16] Guay, J., Bateman, K., Gordon, R., Mancini, J. and Riendeau, D. (2004) Carrageenan-Induced Paw Edema in Rat Elicits a Predominant Prostaglandin E_2 (PGE_2) Response in the Central Nervous System Associated with the Induction of Microsomal PGE_2 Synthase-1. *Journal of Biological Chemistry*, **279**, 24866-24872. https://doi.org/10.1074/jbc.M403106200

[17] Davies, N.M. (1998) Clinical Pharmacokinetics of Ibuprofen. *Clinical Pharmacokinetics*, **34**, 101-154. https://doi.org/10.2165/00003088-199834020-00002

[18] Kim, E.J., *et al.* (2002) Neuroprotective Effects of Prostaglandin E_2 or cAMP against Microglial and Neuronal Free Radical Mediated Toxicity Associated with Inflammation. *Journal of Neuroscience Research*, **70**, 97-107. https://doi.org/10.1002/jnr.10373

[19] Aloisi, F., De Simone, R., Columba-Cabezas, S. and Levi, G. (1999) Opposite Effects of Interferon-γ and Prostaglandin E_2 on Tumor Necrosis Factor and Interleukin-10 Production in Microglia: A Regulatory Loop Controlling Microglia Pro- and Anti-Inflammatory Activities. *Journal of Neuroscience Research*, **56**, 571-580. https://doi.org/10.1002/(SICI)1097-4547(19990615)56:6<571::AID-JNR3>3.0.CO;2-P

[20] Levi, G., Minghetti, L. and Aloisi, F. (1998) Regulation of Prostanoid Synthesis in Microglial Cells and Effects of Prostaglandin E_2 on Microglial Functions. *Biochimie*, **80**, 899-904. https://doi.org/10.1016/S0300-9084(00)88886-0

[21] Degraaf, A.J., Zasłona, Z., Bourdonnay, E. and Peters-Golden, M. (2014) Prostaglandin E_2 Reduces Toll-Like Receptor 4 Expression in Alveolar Macrophages by Inhibition of Translation. *American Journal of Respiratory Cell and Molecular Biology*, **51**, 242-250. https://doi.org/10.1165/rcmb.2013-0495OC

[22] Matsuoka, Y., *et al.* (2005) Prostaglandin E Receptor EP1 Controls Impulsive Behavior under Stress. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 16066-16071. https://doi.org/10.1073/pnas.0504908102

[23] Fritz, M., *et al.* (2016) Prostaglandin-Dependent Modulation of Dopaminergic Neurotransmission Elicits Inflammation-Induced Aversion in Mice. *The Journal of Clinical Investigation*, **126**, 695-705. https://doi.org/10.1172/JCI83844

[24] Fornari, R.V., *et al.* (2012) Rodent Stereotaxic Surgery and Animal Welfare Outcome Improvements for Behavioral Neuroscience. *JoVE* (*Journal of Visualized Experiments*), **59**, e3528. https://doi.org/10.3791/3528

[25] Paxinos, G. and Watson, C. (2006) The Rat Brain in Stereotaxic Coordinates. Elsevier, Amsterdam.

[26] Tomazi, L., *et al.* (2017) A Nonrewarding NMDA Receptor Antagonist Impairs the Acquisition, Consolidation, and Expression of Morphine Conditioned Place Preference in Mice. *Molecular Neurobiology*, **54**, 710-721. https://doi.org/10.1007/s12035-015-9678-0

[27] Prus, A.J., James, J.R. and Rosecrans, J.A. (2009) Conditioned Place Preference. In: Buccafusco, J.J., Ed., *Methods of Behavior Analysis in Neuroscience*, 2nd Edition, CRC Press/Taylor & Francis, Boca Raton, FL, 59-76.

[28] Li, P., *et al.* (2018) AH6809 Decreases Production of Inflammatory Mediators by PGE_2-EP2-cAMP Signaling Pathway in an Experimentally Induced Pure Cerebral Concussion in Rats. *Brain Research*, **1698**, 11-28.

https://doi.org/10.1016/j.brainres.2018.05.030

[29] Wang, X., *et al.* (2012) Morphine Activates Neuroinflammation in a Manner Parallel to Endotoxin. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 6325-6330. https://doi.org/10.1073/pnas.1200130109

[30] Maher, D.P., Walia, D. and Heller, N.M. (2020) Morphine Decreases the Function of Primary Human Natural Killer Cells by Both TLR4 and Opioid Receptor Signaling. *Brain*, *Behavior*, *and Immunity*, **83**, 298-302. https://doi.org/10.1016/j.bbi.2019.10.011

[31] Bland, S.T., *et al.* (2009) The Glial Activation Inhibitor AV411 Reduces Morphine-Induced Nucleus Accumbens Dopamine Release. *Brain*, *Behavior*, *and Immunity*, **23**, 492-497. https://doi.org/10.1016/j.bbi.2009.01.014

[32] Hutchinson, M.R., *et al.* (2008) Non-Stereoselective Reversal of Neuropathic Pain by Naloxone and Naltrexone: Involvement of Toll-Like Receptor 4 (TLR4). *European Journal of Neuroscience*, **28**, 20-29. https://doi.org/10.1111/j.1460-9568.2008.06321.x

[33] Kim, S.-H., *et al.* (2011) Distinct Protein Kinase A Anchoring Proteins Direct Prostaglandin E_2 Modulation of Toll-Like Receptor Signaling in Alveolar Macrophages. *Journal of Biological Chemistry*, **286**, 8875-8883. https://doi.org/10.1074/jbc.M110.187815

[34] Degenhardt, L., *et al.* (2014) The Global Epidemiology and Burden of Opioid Dependence: Results from the Global Burden of Disease 2010 Study. *Addiction*, **109**, 1320-1333. https://doi.org/10.1111/add.12551