Journal of Integrative Neuroscience

J. Integr. Neurosci. **2025**; 24(10): 44311
https://doi.org/10.31083/JIN44311

Original Research

Dopaminergic and Opioid Systems Interact to Produce Peripheral Antinociception in Mice

Bárbara F. G. Queiroz[1], Walace C. P. Barra[1], Flávia C. S. Fonseca[1], Audrey L. Irie[1], Thiago R. L. Romero[1], Igor D. G. Duarte[1,*]

[1]Laboratory of Pain and Analgesia, Department of Pharmacology, Institute of Biological Sciences, Federal University of Minas Gerais, 31270901 Belo Horizonte, Minas Gerais, Brazil

*Correspondence: dimitri@icb.ufmg.br (Igor D. G. Duarte)

Academic Editor: Bettina Platt

Submitted: 27 June 2025 Revised: 29 August 2025 Accepted: 23 September 2025 Published: 28 October 2025

Abstract

Background: The overall pain experience results from the balance between the nociceptive pathway and the body's endogenous modulation of nociception. The interaction of these systems reduces nociception. Therefore, this study aimed to evaluate how the opioid and dopaminergic systems collaborate to inhibit pain at the peripheral level. **Methods**: Swiss mice (30–40 g) had their pain sensitivity increased through paw administration of the prostaglandin E2 (2 µg). They then received opioid and dopaminergic receptor antagonists and agonists, along with an inhibitor of endogenous opioid peptide degradation and a dopamine (DA) reuptake inhibitor. The nociceptive threshold was measured using the paw withdrawal test. Groups were compared using one-way analysis of variance (ANOVA), with $p < 0.05$ considered significant. **Results**: The nonselective opioid receptor antagonist naloxone (50 µg/paw) and the selective κ nor-BNI (200 µg/paw; nor-Binaltorphimine) and δ naltrindole (60 µg/paw) receptor antagonists reversed the antinociception caused by peripheral administration of DA (80 ng/paw), but not the µ-opioid receptor antagonist CTOP (20 µg/paw; D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2). The antinociception induced by a submaximal dose of DA (5 ng/paw) was enhanced by bestatin (400 µg/paw), an inhibitor of endogenous opioid peptide degradation. Conversely, peripheral antinociception from submaximal doses of the µ-, δ-, and κ-opioid agonists DAMGO (0.25 µg/paw; [D-Ala^2, N-Me-Phe^4, Gly^5-ol]-Enkephalin), SNC 80 (5 µg/paw; (+)-4-[(αR)-α-((2*S*,5*R*)-4-Allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide), and bremazocine (200 ng/paw) was increased by the DA reuptake inhibitor GBR 12783 (16 µg/paw; 1-(2-Diphenylmethoxyethyl)-4-(3-phenyl-2-propenyl)-piperazine). Furthermore, the antinociception from these agonists' maximum doses was reversed by dopaminergic D2 (remoxipride, 4 µg/paw) and D3 (U99194, 16 µg/paw; 2,3-Dihydro-5,6-dimethoxy-*N*, *N*-dipropyl-1*H*-inden-2-amine) receptor antagonists, but not by the D4 (L-745, 870, 16 µg/paw; 3-(4-[4-Chlorophenyl]piperazin-1-yl)-methyl-1*H*-pyrrolo[2,3-*b*]pyridine trihydrochloride) receptor antagonist. **Conclusions**: Overall, the data suggest that opioid-mediated antinociception depends on the activation of the dopaminergic system. This demonstrates that pain modulation can be enhanced through the interaction of these systems. Controlling pain at a peripheral level by activating endogenous pathways could be a promising approach to pain management.

Keywords: peripheral antinociception; dopamine; opioid; pain modulation

1. Introduction

The opioid system is a key pain-relief mechanism that functions by inhibiting pain signals. It involves three receptor types: µ, δ, and κ. When these receptors are activated, they reduce neuronal excitability, block the potential of action transmission, and prevent the release of excitatory neurotransmitters, thereby decreasing pain sensation [1–3]. In addition to mechanisms in the brain and spinal cord, opioids can also offer pain relief at the peripheral level [4–6]. Peripheral opioid receptors are located on sensory neurons and interact with both natural and synthetic opioid ligands [7,8].

Although opioid analgesics are some of the most powerful medications for treating severe pain, their long-term use can lead to analgesic tolerance, addictive behaviors, and other adverse effects such as respiratory depression and constipation [9–11]. Therefore, it is essential to find alternative drugs that provide effective pain relief with fewer side effects. A significant number of pre- and clinical investigations highlight the role of the dopaminergic system in modulating nociception. Dysfunction of dopaminergic neurotransmission may be associated with heightened pain sensitivity. Positron emission tomography examinations used to evaluate patients with fibromyalgia syndrome revealed decreased dopamine (DA) synthesis and release in presynaptic neurons [12,13]. Additionally, clinical research indicates that administering levodopa, the precursor of DA, relieves pain in individuals with Parkinson's disease [14,15]. In preclinical trials, injecting levodopa into the intrathecal space and specific regions of the central nervous system produced an antinociceptive effect [16,17]. Peripherally, intraplantar administration of DA reversed Prostaglandin E2-induced hyperalgesia in mice [18].

Recent research indicates that higher endogenous dopamine levels improve the cannabinoid agonist 2-AG (2-Arachidonoylglycerol)-induced peripheral antinociception. It is also important to understand that the interaction between the dopaminergic and endocannabinoid systems plays a key role in the inhibitory regulation of nociception at the peripheral level [19]. Similar to the endocannabinoid system, the opioid system appears to have an additive or synergistic effect with the dopaminergic system in the relief of pain. Activation of μ-opioid receptors in the periaqueductal gray (PAG) depends on the activation of dopaminergic receptors [20]. These receptors are widely found in the ventrolateral PAG and contribute to antinociception caused by systemically administered morphine [21,22].

This study explores whether DA-induced peripheral antinociception involves engaging the opioid system and identifies which opioid agonists and receptors may trigger it. It also investigates if the dopaminergic system plays a role in opioid-mediated peripheral antinociception. The results indicate a possible approach to improve the effectiveness of lower opioid doses in pain management, reducing side effects, while also clarifying the peripheral mechanisms of dopaminergic antinociception.

2. Material and Methods

2.1 Animals

Male Swiss mice (30–40 grams, 10–12 weeks old) from the Bioterism Center (CEBIO) of the Federal University of Minas Gerais (UFMG) were used. The study involved 340 animals, with five mice in each group (sample size of 5). The animals were housed in open-top cages (OTCs) in groups of five per cage, using plastic cages measuring 41 × 33 × 18 cm with sawdust bedding. They were maintained at controlled humidity levels (between 40% and 60%) and a temperature (23 ± 1 °C), under a light/dark cycle (6 AM–6 PM), with free access to water and rodent chow (Nuvilab, Brazil). The Animal Experimentation Ethics Committee approved this study under protocols 196/2018 and 129/2021, and we followed ARRIVE 2.0 guidelines. At the end of the experiments, the animals were euthanized with an intraperitoneal overdose of xylazine (Syntec, Santana Parnaiba, SP, Brazil, 30 mg/kg) and ketamine (Syntec; 300 mg/kg); death was confirmed by cervical dislocation.

2.2 Drugs

Hyperalgesic: Prostaglandin E2 (PGE2; Sigma, P-0409, St. Louis, MO, USA; 2 μg/paw) dissolved in 10% ethanol in saline (NaCl 0.9%); **opioid system drugs:** Naloxone (NX; Sigma, N-109; 12.5, 25, and 50 μg/paw) non-selective opioid receptor antagonist, dissolved in saline; CTOP (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2; Tocris, 1578, Bristol, UK; 20 μg/paw) selective μ-opioid receptor antagonist, dissolved in saline; Naltrindole (NTD; Tocris, 0740; 15, 30, and 60 μg/paw) selective δ-opioid receptor antagonist, dissolved in saline; Norbinaltorphimine (nor-BNI; Tocris, 0347; 50, 100, and 200 μg/paw) selective κ-opioid receptor antagonist, dissolved in DMSO (Dimethyl sulfoxide (Syntech, 01D1011.01.BJ, Syntech, Diadema, SP, Brazil, 10% in saline); Bestatin (Best; Tocris, 1956; 400 μg/paw) inhibitor of the enzyme that degrades endogenous opioid peptides, dissolved in saline; DAMGO (Sigma, E7384; 0.25 and 1 μg/paw) selective κ-opioid receptor agonist, diss/olved in saline; SNC 80 (Tocris, 0764; 5 and 20 μg/paw) δ-opioid receptor agonist, dissolved in DMSO (10% in saline); Bremazocine (Bre; RBI, 74100-60-0, Natic, MA, USA; 200 and 600 ng/paw) κ-opioid receptor agonist, dissolved in saline; **dopaminergic system drugs:** Dopamine (DA; 5, 80 ng/paw, from Sigma, H8502) dissolved in saline; GBR 12783 (16 μg/paw, from Tocris, 0513) dopamine reuptake inhibitor, dissoved in saline; dopaminergic receptor antagonists: D2, D3, and D4, respectively-Remoxipride (Remo; Tocris, 0916; 4 μg/paw), U 99194 (Tocris, 1357; 16 μg/paw), and L-745, 870 (Tocris, 1002; 16 μg/paw), dissolved in saline. The drugs were administered into the right hind paw of the mice at doses that caused only peripheral effects. A volume of 20 μL per paw was used for each drug using insulin needles (30G). All doses and effect timings of the drugs were determined through pilot experiments or data from the literature [18,19,23,24].

2.3 Nociceptive Test

The pressure paw withdrawal method was used to determine the nociceptive threshold [25,26]. During testing, mice were placed on their ventral side on a table, with the paw being tested positioned so that its plantar surface rested on the compressive surface of the analgesimeter (37215, 37216, Ugo-Basile, Gemonio, VA, Italy). This device applied gradually increasing pressure (grams/second) until the paw was withdrawn. The pressure (grams) at which the paw withdrew was defined as the mechanical nociceptive threshold. A cutoff of 150 grams was set to prevent potential injury. The threshold was calculated by averaging three consecutive measurements. The Δ nociceptive threshold was obtained by subtracting the baseline measurement (pre-drug) from the post-drug measurement. Mice were adapted to the analgesimeter for 2 days before testing under the same conditions as during the experiment.

2.4 Experimental Protocol

Initially, the nociceptive threshold was measured before administering any drug (basal) using the paw pressure test. At 0 h, hyperalgesic Prostaglandin E2 (PGE2) was administered. Literature indicates that PGE2 hyperalgesia peaks at 180 minutes after administration [27]. Therefore, all other drugs were given so their peak effects aligned with this time, when the nociceptive threshold was measured again (180 minutes). The analysis focused on the change (Δ) in nociceptive threshold, which is the differ-

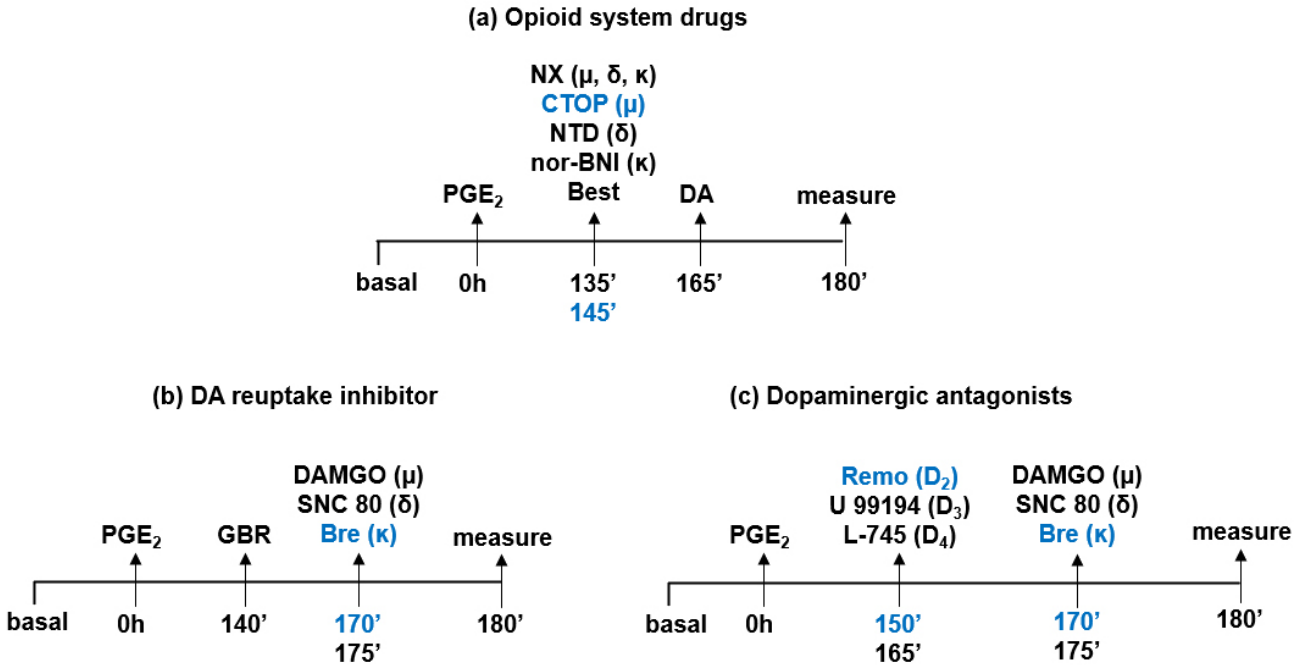

Fig. 1. Timeline of experiments. The baseline nociceptive threshold was measured before administering any drug. All drugs were injected into the right hind paw via intraplantar injection. Prostaglandin E2 (PGE2; 2 μg) was injected into the paw at 0 hours. The final nociceptive threshold was measured after 180 minutes. (a) Naloxone (NX; 12.5, 25, and 50 μg/paw), naltrindole (NTD; 15, 30, and 60 μg/paw), and nor-BNI (50, 100, and 200 μg/paw) were administered along with a maximum dose of dopamine (DA; 80 ng/paw). Bestatin (Best; 400 μg/paw) was administered with a submaximal dose of DA (5 ng/paw). (b) GBR 12783 (16 μg/paw) was administered alongside submaximal doses of DAMGO (0.25 μg/paw), SNC 80 (5 μg/paw), and bremazocine (Bre; 200 ng/paw). (c) Remoxipride (Remo; 4 μg/paw), U 99194 (16 μg/paw), and L-745,870 (16 μg/paw) were administered together with a submaximal dose of DAMGO (1 μg/paw), SNC 80 (20 μg/paw), and Bre (600 ng/paw).

ence between the baseline (0 h) and the value at 180 minutes. All drugs were injected into the right hind paw (intraplantar) in a volume of 20 μL per paw, at doses causing only local effects. These doses were based on the literature [18,19,23,24] and pilot experiments. In the first phase, the role of the opioid system in peripheral DA antinociception was examined. PGE2 was administered at zero h, followed by opioid receptor antagonists—NX, NTD, and nor-BNI—at various doses at 135 minutes. The CTOP μ-opioid antagonist was given at 145 minutes, DA (at its maximum dose) at 165 minutes, and the nociceptive threshold was measured (180 minutes). Bestatin (Best), an inhibitor of endogenous opioid degradation, was given at 135 minutes, and DA (at a submaximal dose capable of being potentiated) at 165 minutes, with the threshold measured at 180 minutes (Fig. 1a). In the second phase, the involvement of the dopaminergic system in opioid-mediated antinociception was tested. PGE2 was administered at 0 h, the DA reuptake inhibitor GBR was given at 140 minutes, and opioid agonists DAMGO and SNC 80 (at submaximal doses) at 175 minutes. Bremazocine (Bre; at a submaximal dose) was administered at 170 minutes, and the threshold was measured at 180 minutes (Fig. 1b). Dopamine receptor antagonists remoxipride (remo), U 99194, and L-745,870 were given at 150, 165, and 165 minutes, respectively. Subsequently, the opioid agonists were administered at their maximum doses at designated times, and the threshold was evaluated at 180 minutes (Fig. 1c). The timelines for drug administration and experiment procedures are shown in Fig. 1.

2.5 Statistical Analysis

Data were analyzed with Prism software version 8.0.2 (GraphPad, Boston, MA, USA), and normality was confirmed using the Shapiro-Wilk test. Our primary outcome variable was continuous; therefore, we compared means between the control and treated groups using one-way analysis of variance (ANOVA). Pairwise comparisons were assessed with a Bonferroni post hoc correction for multiple comparisons, and effects were expressed as mean difference ± 95% confidence interval. Results were presented as mean ± standard deviation (SD) for each experimental protocol with n = 5 animals. Only p-values < 0.05 were considered statistically significant. All tests were one-tailed.

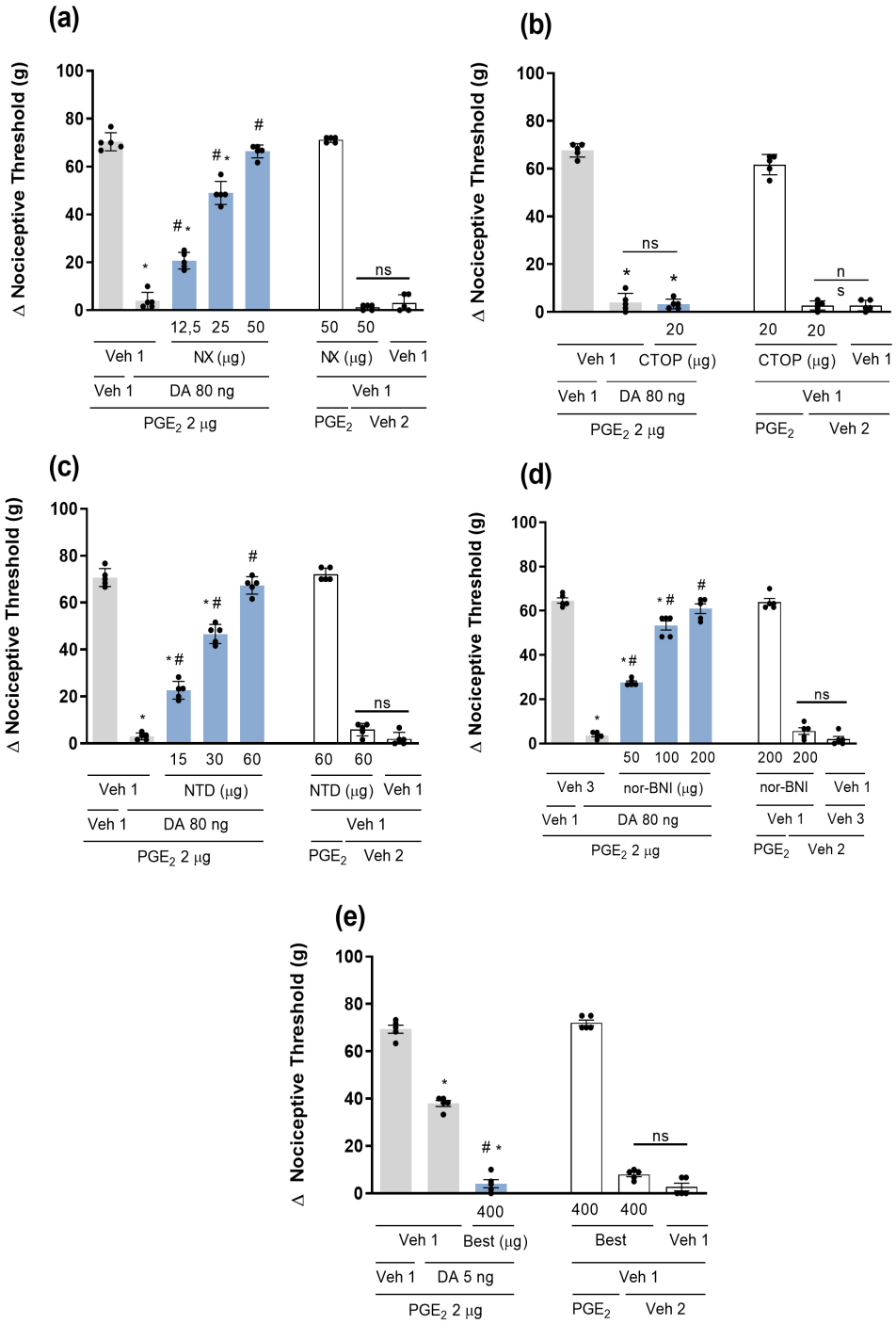

Fig. 2. Participation of the opioid system in dopamine-mediated peripheral antinociception. Effect of both nonselective and selective antagonists for μ, δ, and κ opioid receptors, respectively. (a) Naloxone (NX; 12.5, 25, and 50 μg/paw), (b) CTOP (20 μg/paw), (c) naltrindole (NTD; 15, 30, and 60 μg/paw), and (d) nor-BNI (50, 100, and 200 μg/paw). Additionally, the effect of the endogenous opioid peptide degradation inhibitor (e) Bestatin (Best; 400 μg/paw) on DA-mediated peripheral antinociception was examined. PGE2 (2 μg/paw), opioid system drugs, and DA (5 or 80 ng/paw) were administered at specific times. The nociceptive threshold was measured at 180 minutes. * and # indicate statistically significant differences ($p < 0.0001$; ANOVA followed by Bonferroni test) between the PGE2 2 μg + Veh + Veh and PGE2 2 μg + Veh + DA groups, respectively. When administered alone, opioid system drugs did not cause hyperalgesia or antinociception (columns to the right of each graph). ns, not statistically significant; Veh 1 = saline (0.9% NaCl); Veh 2 = 10% ethanol in saline; Veh 3 = 10% DMSO in saline. (a) F (7, 32) = 485.5; (b) F (5, 24) = 567.3; (c) F (7, 32) = 473.0; (d) F (7, 32) = 376.9; (e) F (5, 24) = 508.6.

3. Results

3.1 Participation of the Opioid System in Dopamine-Mediated Peripheral Antinociception

To assess the role of the opioid system in DA-induced antinociception, animals were initially pre-sensitized with intraplantar injections of the hyperalgesic agent Prostaglandin E2 (PGE2; 2 µg/paw). The administration of DA (80 ng/paw) reversed hyperalgesia and restored the nociceptive threshold to baseline levels. DA-mediated antinociception was reversed by naloxone (NX; 12.5, 25, and 50 µg/paw), a non-selective opioid receptor antagonist, in a dose-dependent manner (Fig. 2a). Similarly, naltrindole (NTD; 15, 30, and 60 µg/paw), a selective δ receptor antagonist, and nor-binaltorphimine (nor-BNI; 50, 100, and 200 µg/paw), a selective κ receptor antagonist, also reversed DA-mediated antinociception in a dose-dependent manner (Fig. 2c,d). Conversely, CTOP (20 µg/paw), a selective µ-opioid receptor antagonist, did not affect DA-mediated antinociception (Fig. 2b). None of the antagonists alone caused antinociceptive or nociceptive effects, as shown in the right panels of each graph. Bestatin (Best; 400 µg/paw), an enzyme inhibitor that degrades endogenous opioid peptides, enhanced the intermediate antinociception caused by a submaximal dose of DA (5 ng/paw). The dose of Bestatin used did not produce significant antinociception when administered alone without DA in response to PGE2 hyperalgesia (Fig. 2e).

3.2 Role of the Dopaminergic System in Opioid-Induced Peripheral Antinociception

To investigate the role of endogenous DA in opioid-induced antinociception, it was used a dopamine reuptake inhibitor GBR 12783. The results show that PGE2-induced hyperalgesia (2 µg/paw) was partially reversed by administering submaximal doses of the µ-, δ-, and κ-opioid agonists DAMGO (0.25 µg/paw), SNC 80 (5 µg/paw), and bremazocine (Bre; 200 ng/paw), respectively. When GBR 12783 (16 µg/paw) was administered, it enhanced opioid-mediated antinociception (Fig. 3a). Subsequently, the involvement of dopaminergic receptors in opioid antinociception was examined. The maximum doses of DAMGO (1 µg/paw; Fig. 3b), SNC 80 (20 µg/paw; Fig. 3c), and bremazocine (600 ng/paw; Fig. 3d) produced antinociception that was reversed by D2 and D3 receptor antagonists, remoxipride (4 µg/paw) and U 99194 (16 µg/paw). In contrast, the D4 receptor antagonist L-745,870 had no effect on the nociceptive threshold. Fig. 3e,f show that, when administered alone, the dopaminergic agents and their vehicles did not affect the nociceptive threshold.

4. Discussion

This study used a pharmacological approach to clarify how DA and opioids promote peripheral antinociception by examining how these two systems interact in suppressing pain at the peripheral level. The increase in nociceptive response was caused by PGE2, which sensitizes primary afferent neurons and leads to hyperalgesia to mechanical stimulation [27]. Previous studies have shown that DA blocks the effects of PGE2 in this model by activating D2 family receptors [18]. In addition to the classic roles assigned to the dopaminergic system [28], scientific literature increasingly emphasizes DA's role in pain modulation [5,29,30]. Meanwhile, opioid drugs are well-established in medicine for treating severe pain. Although highly effective, these drugs are linked to analgesic tolerance, addiction, and physical dependence [11]. Therefore, developing new drugs with good analgesic effects and fewer side effects, or drugs that can enhance existing analgesics' effectiveness while reducing adverse effects, is a promising strategy.

Although dopaminergic analgesia is well documented, its mechanisms are not fully understood. Therefore, our study aimed to examine the influence of the opioid system on DA-mediated antinociception at the peripheral level. The interaction between the opioid and dopaminergic systems in producing analgesia in the central nervous system has already been described, as these systems are closely connected anatomically [31]. The periaqueductal gray (PAG) plays a key role in nociception, often linked to opioid activity [32]. Dopaminergic neurons in this region may be involved in opioid analgesia [33]. Additionally, antinociception caused by activating D2 receptors in the PAG requires the activation of opioid receptors [20].

At the peripheral level, we observed that antinociception mediated by DA administration was reversed by antagonism of δ and κ opioid receptors, but not by blocking the µ receptor, demonstrating that dopaminergic antinociception does not depend on activation of this receptor. In a similar study, peripheral antinociception induced by the antipsychotic aripiprazole, a partial agonist of dopamine D2 receptors, was reversed by naloxone and naltrindole, non-selective and selective δ-opioid receptor antagonists, respectively, but not by nor-BNI, a κ-opioid antagonist [34]. Consistent with our findings, blocking µ-opioid receptors did not affect aripiprazole's antinociceptive effects. It is important to note that most opioids used clinically are µ-opioid receptor agonists, and the main adverse effects of this drug class are related to activating this receptor [11]. Therefore, our results indicate that dopaminergic antinociception involves activating the opioid system, but without the adverse effects linked to µ-opioid receptor activation.

Another important finding from our study was that intermediate antinociception caused by DA was enhanced by bestatin, a drug that prevents the breakdown of endogenous opioids. Since different opioid peptides prefer specific receptors, we can infer from our results that, in our model, the opioids involved in dopaminergic antinociception are likely enkephalins and dynorphins, because these peptides can activate δ and κ-opioid receptors [11,35], which are involved in DA antinociception. By exclusion, since µ-type receptors were not involved, β-endorphin does not seem to be related to this effect.

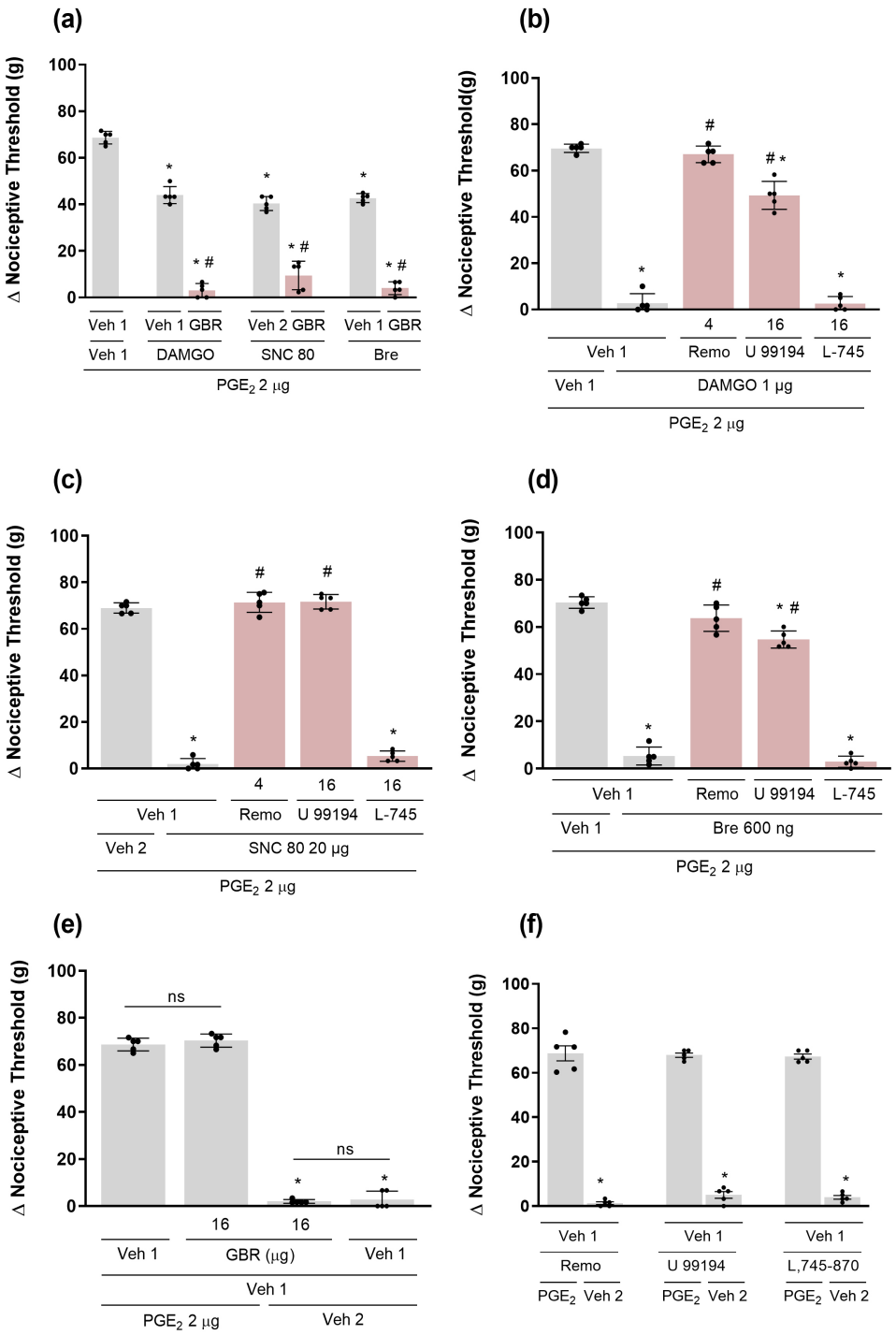

Fig. 3. Involvement of the dopaminergic system in peripheral opioid effects antinociception. Effect of a dopamine reuptake inhibitor (a) GBR 12783 (16 µg/paw) and dopamine receptor antagonists D_2 Remoxipride (Remo; 4 µg/paw), D_3 U 99194 (16 µg/paw), and D_4 L-745,870 (16 µg/paw) on antinociception mediated by µ, δ, and κ opioid agonists, respectively, (b) DAMGO (1 µg/paw), (c) SNC 80 (20 µg/paw), and (d) Bremazocine (Bre; 600 ng/paw) against hyperalgesia induced by PGE2. PGE2 (2 µg/paw), opioid agonists, and dopaminergic drugs were administered at their respective times. The pain pressure threshold was measured at 180 minutes. * and # indicate statistically significant differences ($p < 0.0001$; ANOVA followed by Bonferroni test) compared to the PGE2 2 µg + Veh + Veh or PGE2 2 µg + Veh + opioid agonist groups, respectively. When administered alone, dopaminergic system drugs did not cause hyperalgesia or antinociception (e,f). ns, not statistically significant; Veh 1 = saline (0.9% NaCl); Veh 2 = 10% ethanol in saline. (a) F (6, 28) = 252.8; (b) F (4, 20) = 352.6; (c) F (4, 20) = 774.2; (d) F (4, 20) = 379.0; (e) F (3, 16) = 1033; (f) F (5, 24) = 439.0.

In the next part of our study, we explored how endogenous DA influences opioid antinociception. The opioid system operates through three receptor types: μ, δ, and κ, which can produce analgesia not only via supraspinal and spinal mechanisms but also at the peripheral level [3,7,8]. To evaluate the opioid system, we used the agonists DAMGO, SNC 80, and bremazocine. Previous research has assessed the peripheral antinociceptive effects of these agonists in hyperalgesia models induced by PGE2 or carrageenan [23,36,37], demonstrating that their antinociception depends on activating the noradrenergic system, along with the L-arginine/NO/cGMP pathway. Our findings showed that submaximal doses of these agonists caused moderate antinociception, which was enhanced by increasing endogenous DA availability (by inhibiting its reuptake with GBR 12783). Additionally, dopamine D2 and D3 receptor antagonists, remoxipride and U 99194, reversed the antinociceptive effects of the highest doses of these opioids. Consistent with our results, other studies suggest that D2 receptors interact with opioid receptors and can amplify the analgesic effects of μ-opioid agonists [38]. Moreover, administration of quinpirole, a nonselective D2 family receptor agonist, increased the antinociceptive effect of DAMGO in models of inflammatory and neuropathic pain [39]. Furthermore, systemic delivery of R-VK4-40, a selective D3 receptor antagonist, reduced oxycodone's tolerance and dependence issues (with oxycodone being the most commonly prescribed opioid of abuse) without diminishing its pain-relieving effects [40].

The results of this study, along with the literature review, highlight the critical role of the dopaminergic system in pain modulation, both centrally and peripherally. Additionally, considering the additive and/or synergistic interactions between D2 receptors and opioid receptors, D2 agonists may serve as adjuvants to enhance analgesic effects and decrease the adverse effects of opioids. Overall, these findings suggest that targeting the dopaminergic system could offer a fresh approach for more effective management of pain. This study has some limitations. First, only the mechanical withdrawal test was employed, and additional methods such as the thermal test were not included. Second, only male animals were used in the present experiments. Potential sex differences, as well as the inclusion of additional behavioral tests, will be considered in future studies.

5. Conclusions

This study showed how the body's own pain relief systems work together to influence the blocking of pain signals at the peripheral site. Our data suggest that the opioid system is involved in the peripheral pain relief caused by DA, and that the dopaminergic system contributes to the analgesia produced by opioids. Understanding these mechanisms provides a promising path for creating new treatments that could be more effective than traditional options.

Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

BFGQ: Investigation, Methodology, Writing – original draft; WCPB: Investigation, Methodology; ALI: Investigation, Methodology; FCSF: Investigation, Methodology; TRLR: Investigation, Formal analysis, Resources; IDGD: Investigation, Resources, Formal analysis, Conceptualization, Writing review & editing, Supervision. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The Animal Experimentation Ethics Committee at the Federal University of Minas Gerais approved this study under protocol numbers 196/2018 and 129/2021, and followed ARRIVE 2.0 guidelines. Use Committee and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Acknowledgment

Not applicable.

Funding

This work was supported by grants from Comissão de Aperfeiçoamento de Pessoal do Nível Superior (CAPES), Conselho Nacional de Pesquisa (CNPq), and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Pert CB, Snyder SH. Properties of opiate-receptor binding in rat brain. Proceedings of the National Academy of Sciences of the United States of America. 1973; 70: 2243–2247. https://doi.org/10.1073/pnas.70.8.2243.

[2] Nockemann D, Rouault M, Labuz D, Hublitz P, McKnelly K, Reis FC, *et al*. The K(+) channel GIRK2 is both necessary and sufficient for peripheral opioid-mediated analgesia. EMBO Molecular Medicine. 2013; 5: 1263–1277. https://doi.org/10.1002/emmm.201201980.

[3] Stein C. New concepts in opioid analgesia. Expert Opinion on Investigational Drugs. 2018; 27: 765–775. https://doi.org/10.1080/13543784.2018.1516204.

[4] Picard PR, Tramèr MR, McQuay HJ, Moore RA. Analgesic efficacy of peripheral opioids (all except intra-articular): a qualitative systematic review of randomised controlled trials. Pain. 1997; 72: 309–318. https://doi.org/10.1016/s0304-3959(97)00040-7.

[5] Millan MJ. Descending control of pain. Progress in Neurobiology. 2002; 66: 355–474. https://doi.org/10.1016/s0301-0082(02)00009-6.
[6] Raja SN. Modulating pain in the periphery: gene-based therapies to enhance peripheral opioid analgesia: Bonica lecture, ASRA 2010. Regional Anesthesia and Pain Medicine. 2012; 37: 210–214. https://doi.org/10.1097/AAP.0b013e31823b145f.
[7] Stein C, Hassan AH, Lehrberger K, Giefing J, Yassouridis A. Local analgesic effect of endogenous opioid peptides. Lancet (London, England). 1993; 342: 321–324. https://doi.org/10.1016/0140-6736(93)91471-w.
[8] Stein C. Targeting pain and inflammation by peripherally acting opioids. Frontiers in Pharmacology. 2013; 4: 123. https://doi.org/10.3389/fphar.2013.00123.
[9] Hjsted J, Ekholm O, Kurita GP, Juel K, Sjgren P. Addictive behaviors related to opioid use for chronic pain: a population-based study. Pain. 2013; 154: 2677–2683. https://doi.org/10.1016/j.pain.2013.07.046.
[10] Vadivelu N, Kai AM, Kodumudi V, Sramcik J, Kaye AD. The Opioid Crisis: a Comprehensive Overview. Current Pain and Headache Reports. 2018; 22: 16. https://doi.org/10.1007/s11916-018-0670-z.
[11] Paul AK, Smith CM, Rahmatullah M, Nissapatorn V, Wilairatana P, Spetea M, *et al*. Opioid Analgesia and Opioid-Induced Adverse Effects: A Review. Pharmaceuticals (Basel, Switzerland). 2021; 14: 1091. https://doi.org/10.3390/ph14111091.
[12] Wood PB, Patterson JC, 2nd, Sunderland JJ, Tainter KH, Glabus MF, Lilien DL. Reduced presynaptic dopamine activity in fibromyalgia syndrome demonstrated with positron emission tomography: a pilot study. The Journal of Pain. 2007; 8: 51–58. https://doi.org/10.1016/j.jpain.2006.05.014.
[13] Wood PB, Schweinhardt P, Jaeger E, Dagher A, Hakyemez H, Rabiner EA, *et al*. Fibromyalgia patients show an abnormal dopamine response to pain. The European Journal of Neuroscience. 2007; 25: 3576–3582. https://doi.org/10.1111/j.1460-9568.2007.05623.x.
[14] Nebe A, Ebersbach G. Pain intensity on and off levodopa in patients with Parkinson's disease. Movement Disorders: Official Journal of the Movement Disorder Society. 2009; 24: 1233–1237. https://doi.org/10.1002/mds.22546.
[15] Rabinak CA, Nirenberg MJ. Dopamine agonist withdrawal syndrome in Parkinson disease. Archives of Neurology. 2010; 67: 58–63. https://doi.org/10.1001/archneurol.2009.294.
[16] Dourado M, Cardoso-Cruz H, Monteiro C, Galhardo V. Effect of Motor Impairment on Analgesic Efficacy of Dopamine D2/3 Receptors in a Rat Model of Neuropathy. Journal of Experimental Neuroscience. 2016; 10: 51–57. https://doi.org/10.4137/JEN.S36492.
[17] Cobacho N, De la Calle JL, González-Escalada JR, Paíno CL. Levodopa analgesia in experimental neuropathic pain. Brain Research Bulletin. 2010; 83: 304–309. https://doi.org/10.1016/j.brainresbull.2010.08.012.
[18] Queiroz BFG, Fonseca FCS, Ferreira RCM, Romero TRL, Perez AC, Duarte IDG. Analgesia and pain: Dual effect of dopamine on the peripheral nociceptive system is dependent on D_2-or D_1-like receptor activation. European Journal of Pharmacology. 2022; 922: 174872. https://doi.org/10.1016/j.ejphar.2022.174872.
[19] Gonçalves de Queiroz BF, Cristina de Sousa Fonseca F, Pinto Barra WC, Viana GB, Irie AL, de Castro Perez A, *et al*. Interaction between the dopaminergic and endocannabinoid systems promotes peripheral antinociception. European Journal of Pharmacology. 2025; 987: 177195. https://doi.org/10.1016/j.ejphar.2024.177195.
[20] Tobaldini G, Reis RA, Sardi NF, Lazzarim MK, Tomim DH, Lima MMS, *et al*. Dopaminergic mechanisms in periaqueductal gray-mediated antinociception. Behavioural Pharmacology. 2018; 29: 225–233. https://doi.org/10.1097/FBP.0000000000000346.
[21] Flores JA, El Banoua F, Galán-Rodríguez B, Fernandez-Espejo E. Opiate anti-nociception is attenuated following lesion of large dopamine neurons of the periaqueductal grey: critical role for D1 (not D2) dopamine receptors. Pain. 2004; 110: 205–214. https://doi.org/10.1016/j.pain.2004.03.036.
[22] Voulalas PJ, Ji Y, Jiang L, Asgar J, Ro JY, Masri R. Loss of dopamine D1 receptors and diminished D1/5 receptor-mediated ERK phosphorylation in the periaqueductal gray after spinal cord lesion. Neuroscience. 2017; 343: 94–105. https://doi.org/10.1016/j.neuroscience.2016.11.040.
[23] Romero TRL, Guzzo LS, Duarte IDG. Mu, delta, and kappa opioid receptor agonists induce peripheral antinociception by activation of endogenous noradrenergic system. Journal of Neuroscience Research. 2012; 90: 1654–1661. https://doi.org/10.1002/jnr.23050.
[24] Aguiar DD, Gonzaga ACR, Teófilo ALH, Miranda FA, Perez ADC, Duarte IDG, *et al*. Curcumin induces peripheral antinociception by opioidergic and cannabinoidergic mechanism: Pharmacological evidence. Life Sciences. 2022; 293: 120279. https://doi.org/10.1016/j.lfs.2021.120279.
[25] RANDALL LO, SELITTO JJ. A method for measurement of analgesic activity on inflamed tissue. Archives Internationales De Pharmacodynamie et De Therapie. 1957; 111: 409–419.
[26] Kawabata A, Nishimura Y, Takagi H. L-leucyl-L-arginine, naltrindole and D-arginine block antinociception elicited by L-arginine in mice with carrageenin-induced hyperalgesia. British Journal of Pharmacology. 1992; 107: 1096–1101. https://doi.org/10.1111/j.1476-5381.1992.tb13413.x.
[27] Gonzaga ACR, Quintão JLD, Galdino G, Romero TRL, da Silva GC, Lemos VS, *et al*. Endogenous Cholinergic System Involved in Peripheral Analgesic Control in Mice Is Activated by TNF-α, CXCL-1, and IL-1β. Pharmacology. 2024; 109: 312–329. https://doi.org/10.1159/000538995.
[28] Klein MO, Battagello DS, Cardoso AR, Hauser DN, Bittencourt JC, Correa RG. Dopamine: Functions, Signaling, and Association with Neurological Diseases. Cellular and Molecular Neurobiology. 2019; 39: 31–59. https://doi.org/10.1007/s10571-018-0632-3.
[29] Benarroch EE. Descending monoaminergic pain modulation: bidirectional control and clinical relevance. Neurology. 2008; 71: 217–221. https://doi.org/10.1212/01.wnl.0000318225.51122.63.
[30] Wang XQ, Mokhtari T, Zeng YX, Yue LP, Hu L. The Distinct Functions of Dopaminergic Receptors on Pain Modulation: A Narrative Review. Neural Plasticity. 2021; 2021: 6682275. https://doi.org/10.1155/2021/6682275.
[31] Khachaturian H, Watson SJ. Some perspectives on monoamine-opioid peptide interaction in rat central nervous system. Brain Research Bulletin. 1982; 9: 441–462. https://doi.org/10.1016/0361-9230(82)90154-x.
[32] Bagley EE, Ingram SL. Endogenous opioid peptides in the descending pain modulatory circuit. Neuropharmacology. 2020; 173: 108131. https://doi.org/10.1016/j.neuropharm.2020.108131.
[33] Wood PB. Role of central dopamine in pain and analgesia. Expert Review of Neurotherapeutics. 2008; 8: 781–797. https://doi.org/10.1586/14737175.8.5.781.
[34] Ferreira RCM, Almeida-Santos AF, Duarte IDG, Aguiar DC, Moreira FA, Romero TRL. Peripheral Antinociception Induced by Aripiprazole Is Mediated by the Opioid System. BioMed Research International. 2017; 2017: 8109205. https://doi.org/10.1155/2017/8109205.

[35] Dhawan BN, Cesselin F, Raghubir R, Reisine T, Bradley PB, Portoghese PS, *et al*. International Union of Pharmacology. XII. Classification of opioid receptors. Pharmacological Reviews. 1996; 48: 567–592.

[36] Amarante LH, Duarte IDG. The kappa-opioid agonist (+/-)-bremazocine elicits peripheral antinociception by activation of the L-arginine/nitric oxide/cyclic GMP pathway. European Journal of Pharmacology. 2002; 454: 19–23. https://doi.org/10.1016/s0014-2999(02)02275-6.

[37] Pacheco DF, Reis GML, Francischi JN, Castro MSA, Perez AC, Duarte IDG. delta-Opioid receptor agonist SNC80 elicits peripheral antinociception via delta(1) and delta(2) receptors and activation of the l-arginine/nitric oxide/cyclic GMP pathway. Life Sciences. 2005; 78: 54–60. https://doi.org/10.1016/j.lfs.2005.04.032.

[38] Aira Z, Barrenetxea T, Buesa I, Gómez-Esteban JC, Azkue JJ. Synaptic upregulation and superadditive interaction of dopamine D2- and μ-opioid receptors after peripheral nerve injury. Pain. 2014; 155: 2526–2533. https://doi.org/10.1016/j.pain.2014.09.012.

[39] Mercado-Reyes J, Almanza A, Segura-Chama P, Pellicer F, Mercado F. D2-like receptor agonist synergizes the μ-opioid agonist spinal antinociception in nociceptive, inflammatory and neuropathic models of pain in the rat. European Journal of Pharmacology. 2019; 853: 56–64. https://doi.org/10.1016/j.ejphar.2019.03.020.

[40] Jordan CJ, Humburg B, Rice M, Bi GH, You ZB, Shaik AB, *et al*. The highly selective dopamine D_3R antagonist, R-VK4-40 attenuates oxycodone reward and augments analgesia in rodents. Neuropharmacology. 2019; 158: 107597. https://doi.org/10.1016/j.neuropharm.2019.04.003.