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Opioid antagonism in fentanyl antinociception experimental

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Abstract

Among the most commonly used drugs to reduce pain and inflammation are nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids. Opioids are a wide group of drugs including fentanyl a fully synthetic opioid that is more potent that morphine. The aim of the present study was to evaluate the fentanyl antinociception in two pain murine tests, the acetic acid writhing (WT) and the formalin hind paw (FHP). Likewise, the involvement of opioids antagonists: naltrexone, naltrindole and nor-binaltorphimine in the induced activity of fentanyl was estimated. The antinociceptive activity of fentanyl was evaluated from dose-response curves. The intraperitoneal administration of fentanyl induced a dose related antinociceptive effects with different potencies in both tests. The data revealed a significant decrease in the analgesic effect of fentanyl by action of naltrexone, naltrindole and nor-binaltorphimine in the WT and FHP tests with the exception of nor-binaltorphimine in the WT. The effect of opioids antagonist in reducing the efficacy of fentanyl could possibly be related to other multiple mechanisms added to the inhibition of the activation of the MOR, DOR, and KOR opioid receptors. This study demonstrates that there is a functional interaction modulatory between fentanyl antinociception fentanyl and naltrexone, naltrindole and nor-binaltorphimine in the murine assays of acetic acid writhing and formalin hind paw. This modulation seems to be mediated by the multiple mechanisms of action of fentanyl. These results suggest than fentanyl is able to be effective in models of nociception and the inflammatory pain.

Keywords: Fentanyl; Murine assay; Opioid blockers; NSAIDs

1. Introduction

The most commonly used drugs to reduce pain and inflammation are nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids. However, opioids are among the analgesics of restricted use due to their side effects and potential to induce dependence [1]. Opioids are a wide group of drugs including (a) alkaloids such as morphine, codeine, (b) semisynthetic derivatives: oxycodone, hydromorphone, oxymorphone and (c) synthetic agent such as meperidine, methadone, and fentanyl. These drugs act on the following types of receptors: μ (MOR), δ (DOR), κ (KOR) and ORL1 (or nociceptin, or orphanin FQ) which are widely distributed throughout the central and peripheral nervous system as well as other systems such as the gastrointestinal tract, skin, cardiovascular and immune systems. The widespread distribution of opioid receptors in addition to the analgesics effect also produce a broad spectrum of adverse effects including respiratory depression, nausea, constipation, euphoria, dysphoria, sedation, bradycardia, convulsion, vomiting, pruritus, and miosis [2].

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Opioids exert their antinociceptive effects at presynaptically and postsynaptically level. Thus, presynaptically, blocking calcium channels and then inhibit the release of neurotransmitters such as substance P and glutamate. Postsynaptically, opioid analgesics directly inhibit postsynaptic neuronal activity by hyperpolarizing cell membranes via opening potassium channels [3].

Fentanyl is a synthetic opioid of clinical use since is 50-100 times more potent that morphine and is commonly used for management of severe pain and as an adjunct to general anaesthesia. Fentanyl is an agonist for the MOR opioid receptors has a rapid onset and short duration of action, properties that make it very effective both in the induction and in the maintenance of anesthesia [4-5].

Several investigations have been carried out in animals mostly in rodents, very consistent, aimed at evaluating the fundamental problems of both nociceptive and inflammatory pain. Among them, it can mention the models of acetic acid writhing and formalin hind paw. These assays induce characteristic and quantifiable behavior [6].

The aim of the present study was to evaluate the fentanyl antinociception in two pain murine tests, the acetic acid writhing and the formalin hind paw. In addition, the involvement of opioids antagonists: naltrexone, naltrindole and nor-binaltorphimine in the induced activity of fentanyl was estimated.

2. Material and methods

2.1. Animals

Male CF-1 mice (25-30 g) housed on a 12-hr light–dark cycle at 22 ± 1 °C with free access to food and water were used. Experiments were performed by following current Guidelines for the Care of Laboratory Animals and Ethical Guidelines for investigation of experimental pain approved by the Animal Care and Use Committee at the Faculty of Medicine, Universidad of Chile, protocol N° 238-MED-UCH, 2018. Animals were acclimatized to the laboratory for at least 1 hr before testing, used only once and euthanized by intraperitoneal (i.p.) overdose of anaesthetic immediately after the algesimeter test. The tests were performed by investigators blinded to the treatment of animal and the number of mice was kept at a minimum, compatible with reliable effects of the drug treatment.

2.2. Measurement of antinociception and anti-inflammation

Antinociception was assessed by the following murine assays: (A) acetic acid writhing test (WT) as described previously was used [7]. In this test, the mice were injected i.p. with 10 ml of 0.6% of acetic acid. The chemical stimulus induces a wave of contraction of the abdominal muscle followed by extension of the hindlimbs (writhes) and reduction in motor activity. The number of writhes in 5 min after the i.p. of chemical solution was counted. Antinociception is the percentage of inhibition of the number of writhes in control mice (21.8 ± 1.2, n=24) and converted to % MPE (percentage of maximum possible effect).

(B) the formalin hind paw (FHP) test described previously was used [8]. To perform the test 20 µL of 2 % formalin solution was injected into the dorsal surface of the right hind paw. The intensity of pain was assessed as the time, in sec, by the licking or biting of the injected paw. The test show 2 periods: phase I, corresponding to the 5 min immediately after formalin injection and phase II, chronicled by 10 min, a period starting 20 min after formalin injection. The control values were, phase I: 133.5 ± 7.4 sec, (n =12) and phase II: 157.8 ± 9.2 sec, (n=12). Licking time was converted to % MPE.

2.3. Experimental design

The antinociceptive activity of fentanyl was evaluated from dose-response curves induced by 1, 3,10.30 and 100 mg/kg i.p. for WT and 3,10,30, 100 and 300 for FHP using six to eight animals for each dose. Dose-response were obtained before and after the i.p. administration of 1 mg/kg of naltrexone (NTX), 1 mg/kg of naltrindole (NTI) or 1 mg/kg of nor-binaltorphimine (Nor-BNI). The drugs were administered i.p. 30 min prior to each test. The dose that produces 50 % of the MPE (ED_{50}) was calculated from a linear regression analysis of the dose-response curve. The different doses of drugs used in this work did not induce significant change in behavioural or motor dysfunction in the mice.

2.4. Drugs

All drugs were freshly dissolved in sterile physiological salt solution on a constant volume of 10 ml/kg, for i.p. administration. Fentanyl, naltrexone hydrochloride, naltrindole hydrochloride and nor-binaltorphimine dihydrochloride from Sigma-Aldrich Chemical Co. St. Louis, MO, USA.

2.5. Statistical analysis

Results are presented as mean ± SEM and statistical difference between the results were analyzed by ANOVA followed by Tukey´s post-test and P values less than 0.05 ($P<0.05$) were considered statistically significant. All calculation were performed using the software Pharm Tools Pro, version 1.27, McCary Group Inc., PA, USA.

3. Results

3.1. Antinociception induced by fentanyl

The i.p. administration of fentanyl induced a dose related antinociceptive effects with different potencies in the WT and the FHP tests. Fentanyl developed the highest relative potency, expressed as ED_{50}, in the WT test and the lowest in the FHP-I assay. These results are shown in Table 1 and represented graphically in Figure 1.

3.2. Effect of antagonist's opioid on the antinociception of fentanyl

Mice treated with 1 mg/ kg i.p. of the different opioid antagonist did not exhibit significant differences in pain and locomotor activity compared to controls. To determine the effect of NTX, NTI, and Nor-BNI on the activity of fentanyl in the tests, a dose-response curves was obtained in mice pretreated with each opioid antagonist. The data revealed a significant decrease in the analgesic effect of fentanyl in the WT and FHP tests. However, no significant differences were detected in the WT test due to the action of nor-BNI (see Table 1 and Figure 2).

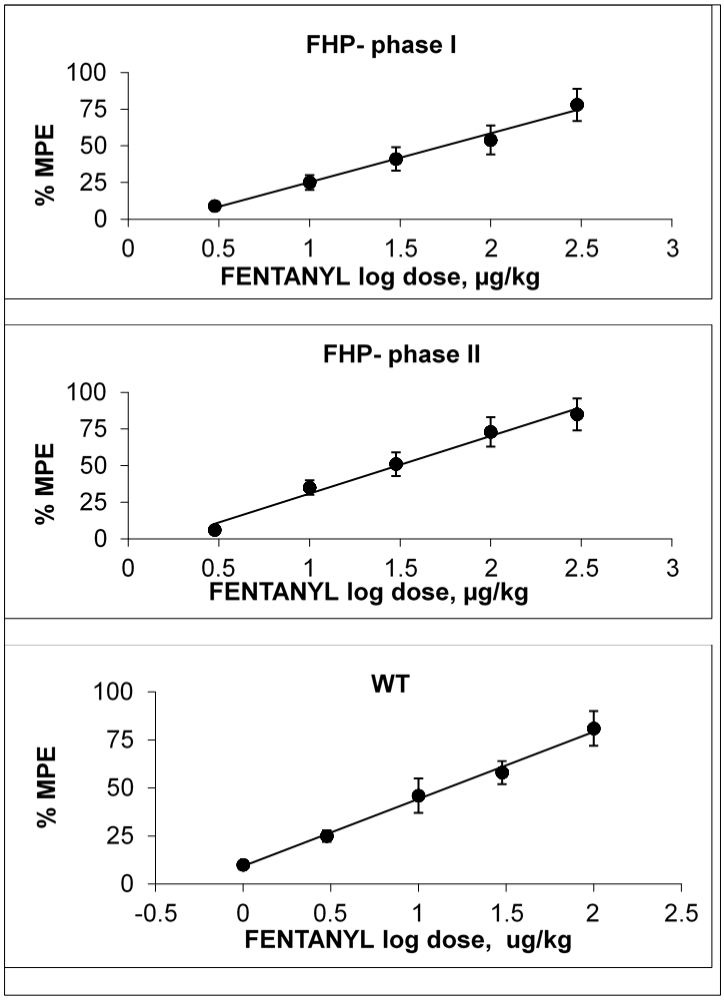

Figure 1 Dose-response curves for the antinociceptive activity induced In mice by intraperitoneal administration of fentanyl in formalin hind paw (FHP) and the acetic acid writhing tests. Each point is the mean ± SEM of 6-8 mice. % MPE: antinociception as percentage of the maximun possible effect

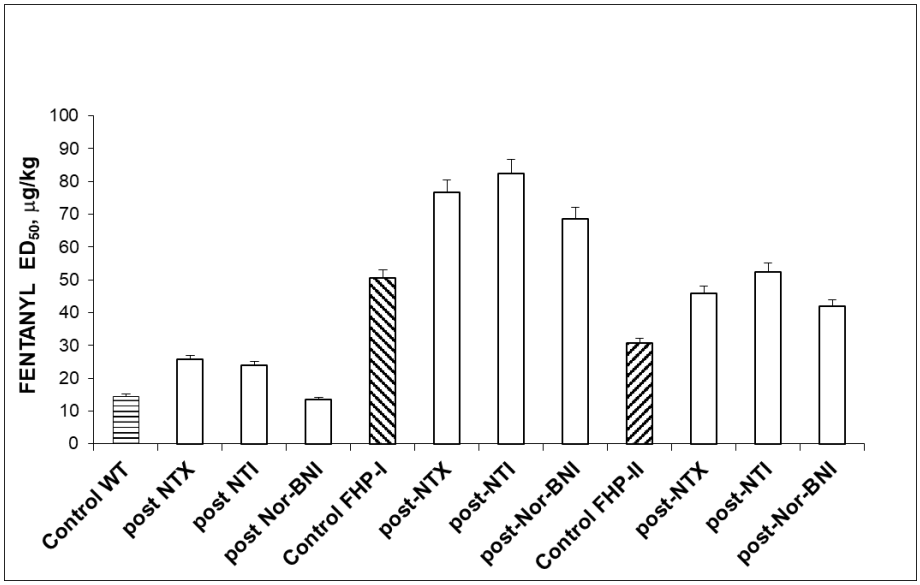

Figure 2 Effect of opioid antagonist's pretreatment on the ED_{50} of fentanyl

Effect of opioid antagonist's pretreatment on the ED_{50} of fentanyl in the acetic acid writhing test (WT) and formalin hind paw, phase I (FHP I) and phase II (FHP II) assays. The ED50 obtained before and after pretreatment is shown in white and black columns, respectively. Columns represent the mean ± SEM of 6–8 mice. *: $P < 0.05$, versus control.

Table 1 ED50 values with SEM in µg/kg for the antinociceptive activity of fentanyl in the acetic acid writhing and formalin hind paw tests of mice before and after treatment with i.p. 1 mg/kg of naltrexone (NTX), 1 mg/kg of naltrindole, (NTI) and 1 mg/kg of Nor-binaltorphimine (Nor-BNI)

Condition	ED50 Control	ED50 post NTX	ED50 post NTI	ED50 post Nor-BNI
FHP-I	50.5 ± 6.7	76.6 ± 6.2	82.5 ± 7.3	68.6 ± 7.8
FHP-II	30.7 ± 3.6	45.9 ± 4.1	52.4 ± 4.6	41.9 ± 8.7
WT	14.5 ± 1.1	25.7 ± 2.1	23.9 ± 2.8	15.1 ± 1.5

FHP-I: formalin hind paw phase I, FHP-II: formalin hind paw phase II, WT: acetic acid writhing test. All E_{D50} post treatment are significant ($P< 0.05$) versus control with the exception of WT post-nor-BNI

In addition, changes in ED50, expressed as the ratio between ED50 values, as analgesia ratio (AR), varied between 1.8 and 1.0, in the following order: NTX > NTI > nor-BNI in WT; NTI > NTX > nor-BNI in FHP II; NTI > NTX > nor-BNI, as shown in Table 2

Table 2 Analgesic ratio (AR: ratio $ED_{50\ post}$/pre-treatment) values for the antinociceptive activity of fentanyl, between ED_{50} pre-/post treatment with NTX, NTI or nor-BNI in mice algesimeter tests

C0NDITION	NTX	NTI	Nor-BNI
WT	1.8	1.6	1.0
FHP-I	1.5	1.6	1.4
FHP-II	1.5	1.7	1.4

WT: acetic acid writhing; FHP I: formalin hind paw, phase I; FHP II: formalin hind paw, phase Il. NTX: naltrexone, NTI: naltrindole; nor-BNI: nor-binaltorphimine

4. Discussion

Opioids are frequently used in the treatment of pain, but their adverse effects restrict their use, which is why an attempt is made to specify the modulation of their mechanism of action in order to be able to use them in multimodal analgesia.

In this work, the modulation of opioid antagonists and fentanyl was evaluated. Dose-dependent antinociceptive activity of fentanyl was found, independent of the nociceptive chemical noxa, inducing tonic pain, whether caused by acetic acid or formalin. Nociceptive efficacy manifests both in nociceptive pain (WT and FHP-phase I) and in inflammatory origin (FHP-phase II). Findings consistent with previously reported results [9-11].

The current study displayed a significant decrease in the fentanyl efficacy in the WT and FHP tests by action of NTX, NTI and nor-BNI, with the exception in the WT test due to the action of nor-BNI. These results demonstrate the modulatory effects of opioid antagonists on nociceptive pain which involves several mediators such as prostanoids, proinflammatory cytokines, interleukin, tumor necrosis factor (TNF)- α, the activation of visceral nociceptors by the action of MOR and they also exhibit effects that include the upregulation of substance P, serotonin, histamine, bradykinin and increase of glutaminergic transmission [12,13].

The effect of NTX, NTI, and nor-BNI in reducing the efficacy of fentanyl could possibly be related to other multiple mechanisms added to the inhibition of the activation of the MOR, DOR, and KOR opioid receptors.

There are other mechanisms of action of fentanyl on which the opioid antagonists could be acting, since the MOR activation induce a decrease in cAMP and inhibits the release of neurotransmitters such as GABA, dopamine, acetylcholine, and noradrenaline [2].

Other mechanisms have been proposed for the hypoalgesic effect of fentanyl in which opioid antagonists could be involved, either directly or indirectly, for example, the results obtained could be explained by the fact that fentanyl is capable of inducing serotonin reuptake inhibition and increased intersynaptic serotonin release by inhibiting gamma-aminobutyric acid (GABA). Furthermore, it binds to the N-methyl-D-aspartate (NMDA) receptor and antagonizes the effect of glutamate [14].

Another possibility to explain the findings of this study with fentanyl could be the antagonism of the adrenergic receptor subtypes (α1B > α1A >>> α1D) that have been demonstrated for the opioid [15].

Another possibility to explain the findings of this study would be to ascribe the participation of opioid antagonists in the efficacy of fentanyl to the selective modulation of the opioid in M_1-M_5 muscarinic receptors [16].

The findings of this study indicate that the antinociceptive effect produced by fentanyl is modulated by naltrexone, naltrindole, and nor-binaltorphimine and that, regardless of their mechanisms of action and the type of pain induced, there is a significant reduction in the antinociceptive and anti-inflammatory effects of fentanyl.

5. Conclusion

This study demonstrates that there is a functional interaction modulatory between fentanyl antinociception fentanyl and naltrexone, naltrindole and nor-binaltorphimine in the murine assays of acetic acid writhing and formalin hind paw. This modulation seems to be mediated by the multiple mechanisms of action of fentanyl. These results suggest than fentanyl is able to be effective in models of nociception and the inflammatory pain.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

Experiments were performed by following current Guidelines for the Care of Laboratory Animals and Ethical Guidelines for investigation of experimental pain approved by the Animal Care and Use Committee at the Faculty of Medicine, Universidad of Chile, protocol N° 238-MED-UCH, 2018.

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Authors' contributions

All authors contributed equally in preparing all parts of the work and approved the version submitted for revision.

References

[1] Manhapra A. Complex Persistent Opioid Dependence-an Opioid-induced Chronic Pain Syndrome. Curr Treat Options Oncol. 2022; 23(7):921-935.

[2] Al-Hasani R, Bruchas MR. Molecular mechanisms of opioid receptor-dependent signalling and behavior. Anesthesiology. 2011;115(6):1363-1381.

[3] Jamison RN, Mao J. Opioid Analgesics. Mayo Clin Proc. 2015;90(7):957-968.

[4] Comer SD, Cahill CM. Fentanyl: Receptor pharmacology, abuse potential, and implications for treatment. Neurosci Biobehav Rev. 2019; 106:49-57.

[5] Armenian P, Vo KT, Barr-Walker J, Lynch KL. Fentanyl, fentanyl analogs and novel synthetic opioids: A comprehensive review. Neuropharmacology. 2018 ;134(Pt A):121-132.

[6] Le Bars D, Gozariu M, Cadden SW. Animal models of nociception. Pharmacol Rev. 2001;53(4):597-652.

[7] Miranda HF, Puig MM, PrietoJ C, Pinardi G. Synergism between paracetamol and nonsteroidal anti-inflammatory drugs in experimental acute pain. Pain 2006; 121: 22-28.

[8] Miranda HF, Noriega V, Olavarria L et al. Antinociception and antiinflammation induced by simvastatin in algesimeter assays in mice. Basic Clin Pharmacol Toxicol 2011;109: 438-442.

[9] Romero A, Miranda HF, Puig MM. Analysis of the opioid-opioid combinations according to the nociceptive stimulus in mice. Pharmacol Res. 2010 ;61(6):511-518.

[10] Miranda HF, Noriega V, Zanetta P, et al. Isobolographic analysis of the opiod-opiod interactions in a tonic and phasic mouse model of induced nociceptive pain. J Biomed Sci. 2014;21: 62-70.

[11] Nakamura Y, Okano Y, Sato M, Kobayashi M, Yamaguchi T, Sumi T, Koyama Y, Kondo M, Usui N, Shimada S. Pain-like behavior in mice can be induced by the environmental context in which the pain stimulus was previously given. Neuroreport. 2021 ;32(5):386-393

[12] Yam MF, Loh YC, Oo CW, Basir R. Overview of neurological mechanism of pain profile used for animal "pain-like" behavioural study with proposed analgesic pathways. Int J Mol Sci. 2020;21:4355-4380.

[13] Seifert O, Baerwald C. Interaction of pain and chronic inflammation. Z Rheumatol. 2021;80(3):205-213.

[14] Baldo BA, Rose MA. The anaesthetist, opioid analgesic drugs, and serotonin toxicity: a mechanistic and clinical review. Br J Anaesth. 2020;124(1):44-62.

[15] Sohn JT, Ding X, McCune DF, Perez DM, Murray PA. Fentanyl attenuates alpha1B-adrenoceptor-mediated pulmonary artery contraction. Anesthesiology. 2005 ;103(2):327-334.

[16] Torralva R, Janowsky A. Noradrenergic mechanisms in fentanyl-mediated rapid death explain naloxone failure in opioid crisis. J Pharmacol Exp Ther. 2019;371(2):453-475