Analgesic Interaction Between Morphine with Fentanyl In Experimental Murine Pain

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Abstract

Opioids are among the most effective pain relievers available, however multimodal antinociception between opioids, has not been extensively studied in diverse animal pain models.In this study the pharmacological interaction of morphine with fentanyl was evaluated in different murine pain models by means of isobolographic analysis. In control animals, morphine and fentanyl produced a dose-related antinociceptive action in the murine assays and the rank of potency was: formalin hind paw phase I > formalin phase II > tail flick. Coadministration of morphine with fentanyl, in a fixed relation 1:1 of their ED_{50}, produces a dose response in all tests and the isobologram resulted in synergism. Fentanyl was more effective than morphine which could be explained according the suggestion that opioids could be acting through other targets, with different binding capacity thru the regulation or activation of non-opioid receptors. Co-administration of morphine with fentanyl induces synergism in all murine trials, confirming the antinociceptive capacity of both opioids which would constitute a promisory idea to multimodal treatment of pain.

Introduction

In the therapy of pain, opioids are among the most effective pain relievers available. These drugs have demonstrated analgesic efficacy in different animal models of pain, including models of tonic pain: acetic acid and formalin writhing test and phasic pain tests: tail movement and hot plate. Opioids comprise a variety of drugs both natural (morphine, codeine) and synthetic (fentanyl, methadone, heroin), all of which are powerful and effective pain relievers, but with high potential for dependency and abuse. Opioid receptors are coupled to G proteins and divided into subtypes: MOR (μ1, μ2, μ3); DOR (δ1, δ2, δ3); and KOR (κ1, κ2, κ3).They are transmembrane domains, three extracellular and three intracellular loops with N-terminal extracellular amino acid terminations and intracellular C-terminal carboxyl. Opioid receptors are found in both the central and peripheral nervous systems and activation of these receptors by ligands induces significant molecular changes inside the cell, such as an inhibition of adenylate cyclase activity, activation of potassium channels and reductions of calcium conductance. In this context, G protein receptor kinases (GRKs) are important modulators of opioid receptor function. Recent data indicate that other signalling pathways also may be involved in morphine activity. Among these are phospholipase C, mitogen-activated kinases (MAP kinases) or β-arrestin. There are endogenous natural ligands for opioid receptors such as β-endorphins, encephalin, dynorphins, and nociceptin/orphanin FQ. Opioids have a variety of effects, including pain relief, euphoria, drowsiness, sedation, dizziness, nausea, vomiting, constipation, physical dependence, tolerance, addiction and respiratory depression (Lemel et al. 2020; Corder et al. 2018).

Opioids can be classified on the basis of their chemical structure as (I) opium alkaloids or opiates: codeine, morphine, (II) semisynthetic derivatives of the natural alkaloids: hydrocodone, hydromorphone, oxycodone, buprenorphine, and (III) synthetic opioids such as fentanyl, alfentanil, methadone, loperamide, tramadol and the opioid antagonists as nalmefene, naloxone, naltrexone, naltrindole, nor-binaltorphimine (Pathan and Williams 2012).

The different opioids, due to their frequent use, deserve to be highlighted: (A) Natural opioid morphine with powerful analgesic effects, used in clinical medicine for almost two hundred years and acts by activating MOR-type receptors in the CNS and PNS. (B) Synthetic opioid fentanyl with pain action similar to morphine, but 50 to 200 times more potent. Like morphine, fentanyl is an agonist of MOR-like receptors in the CNS and PNS. Fentanyl is effective in both induction and maintenance of anesthesia (Pathan and Williams 2012; Corder et al. 2018).

The multimodal analgesia, a method of combining analgesic drugs, is an approach should be used when treating pain reactive to the action of a single analgesic, which leads, for example in the case of opioids, to a potential reduction in undesirable effects such as nausea, vomiting, respiratory depression and also provide relief of pain using different routes of cellular action. When two drugs are taken together if the resulting response can be greater than the sum of the individual response, the interaction between the drugs is synergistic (Tallarida 2001).

In the present study, the pharmacological co-interaction of morphine with fentanyl was evaluated in different murine pain models by means of isobolographic analysis.

Materials And Methods

Animals

Male CF-1 mice (25-30g) housed on a 12 h light-dark cycle at 22±1 °C with free access to food and water ad libitum, were used. All procedures with animals from the central nursery were approved by the Animal Care and Use Committee of the Faculty of Medicine. (Protocol CBA 0852/FMUCH/2018). Animals were acclimatized to the laboratory for at least 1 h before testing, used only once during the protocol, and euthanized after the algesiometric test by one intraperitoneal (i.p.) injection of 60 mg/kg of pentobarbital. The number of animals was kept at a minimum, compatible with consistent effects of the drug treatment.

Measurement of antinociceptive activity

Antinociception was assessed by the following murine tests:

(A) tail-flick test (TF) as described previously (Miranda et al. 2001). A radiant heat, automatic tail flick (Ugo Basile, Comerio, Italy) was used to measure response latencies. Baseline was obtained before protocol and then test latency after administration of drugs. A cut-off time of 8 sec was set to avoid damage of mice tissue. TF latencies controls were 2.65 ± 0.12 (n=12) and converted to % MPE.

(B) the formalin hind paw (FHP) test described previously was used (Miranda et al. 2001). 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: 133.05 ± 7.04 (n =12) and phase II: 157.83 ± 9.10 (n=12). Licking time was converted to % MPE.

Experimental design

The antinociceptive potency of morphine and fentanyl, a dose-response curve from 0.1, 0.3, 1 and 3 mg/kg, i.p. for morphine and 0.03, 0.06, 0.12 and 0.24 mg/kg i.p. for fentanyl was obtained in the WT, TF, FHP and HP tests using at least 6 animals for each at least 4 doses, as can be seen in figure 1. Then the DE_{50}, dose that induce 50% of MPE, was calculated from lineal regression of dose-response curves of morphine and fentanyl.

Isobolographic analysis

The interaction morphine with fentanyl ws evaluated by isobolography (Miranda et al., 2001, 2007, 2012). The isobolograms were constructed connecting ED_{50} of fentanyl in the abscissa with ED_{50} of morphine in the ordinate to obtain the additive line. For each combination, the ED_{50} was obtained and compared with the ED_{50} theoretically and symbolized by a point on the additive line. A synergistic or supraadditive effect is if the ED_{50} experimental is significant lower than the theoretical ED_{50} and represented by a point below the additive line. Also the nature and magnitude of the interaction is indicated the interaction index (I.I.) a the ratio of combination potency, calculated as: [I.I. = experimental ED_{50} / theoretical ED_{50}]. If the value is below 1, the interaction is supraadditive or synergistic.

Drugs

Drugs were freshly dissolved in sterile physiological salt solution of 10 mL/Kg, for i.p. administration. Morphine hydrochloride and fentanyl hydrochloride were obtained from Sigma-Aldrich Chemical Co, St. Louis, Mo, USA.

Statistical analysis

Results are presented as means ± SEM or 95 % confidence limits (95 % CL). The statistical difference between the results were assessed by one-way ANOVA, followed by Tukey's post test for and p values less than 0.05 ($p<0.05$) considered statistically significant. Statistical analyses were carried out using the program Pharm Tools Pro, version 1.27, Mc Cary Group Inc., PA, USA.

Results

Antinociception of morphine and fentanyl.

In control mice, i.p. administration of morphine and fentanyl produced a dose-related antinociceptive action in the FHP and TF assays and the ED_{50} with SEM resultant are shown in Table 1 and Fig.1. The order of analgesic ratio for morphine was: phase I of FHP > phase II of FHP > TF. In fentanyl the order was: phase II of FHP > phase I of FHP > TF. Comparison of the potency of NSAIDs was: phase I of FHP > phase II of PHF > TF. Data in Table 1.

Analysis of interaction morphine with fentanyl

The i.p. coadministration of morphine with fentanyl, in relation 1:1 of their ED_{50}, produces a dose response in all experimental conditions. The isobologram demonstrated that the opioids combination, in the FHP and TF, resulted in a synergistic interaction of different magnitude, as can be seen in Table 2 and Fig.2 . The degree of potency of the mixture, according the interaction index, revealed the following rank: FHP, phase II > TF > FHP, phase I. These changes were accompanied by similar modification of ED_{50} values (see Table 2).

Discussion

Opioids are widely used in the treatment of pain. Opioids activate, at the supraspinal level, the MOR receptors of the GABAergic interneurons of the descending pathway of pain modulation and the dorsal horn of the spinal cord. These GABA changes contribute to opioid-induced antinociception. However, antinociception induced by them has not been extensively studied in different animal pain models. The most of the major studies have been conducted with isolated opioids, but have not been used in combination, as suggested by multimodal analgesia. This study demonstrated that morphine and fentanyl induce antinociception in FHP and HP tests, with different antinociceptive potency, with fentanyl had a higher effect than morphine, in all tests, results consistent with the analgesic properties of fentanyl being approximately 100 times more potent than morphine. Furthermore, fentanyl has a different binding capacity, expressed in nmol, from 0.7 to 1.9, compared to morphine from 1.02 to 4 (Zaveri et al. 2001; Waldhoer et al. 2004).

The present results are in agreement with previous studies, which demonstrate that morphine and fentanyl induce analgesia in different animal pain tests, such as acetic acid writhing, formalin hind paw, hot plate and tail flick assays (Miranda et al., 2001, 2007, 2012; Romero et al. 2010). Co-administration of morphine with fentanyl demonstrated synergism in the FHP and TF assays being more powerful in the FHP-I and less potent in phase II of FHP. These results are in agreement with the general basis of synergism which propose that the effect occurs when two drugs are co-administered with same effect but have a different mechanism of action. In the present work, the interaction could be attributed to various levels of cellular function, among which pain receptors, messengers or mediators can be mentioned.

Opioids are known to interact with specific receptors (MOR, DOR, KOR, NOP) with different selectivity and pharmacological and biochemical studies have demonstrated the existence of modulatory interactions between opioid and receptors. It has been suggested that morphine antinociception is preferentially mediated through MOR and it is believed that most clinical opioids exert their antinociceptive effects through MOR. Opioids may show different efficiencies probably due to MOR receptor subtypes, since 5 splice variants of the mouse-MOR have been described, so as functional consequences, they could explain the greater efficacy of fentanyl over morphine (Pasternak 2004; Anathan 2006).

The study shows that fentanyl is a more effective antinociceptive opioid than morphine. This finding is consistent with a previous study that revealed different mechanisms for the antinociception of morphine and fentanyl (Morgan et al. 2020). Furthermore, it could be explained by the suggestion that some

opioids could be acting through other mechanisms. Thus, a multi-opioid study using radioligand binding and functional activity assays found novel interactions, including activation of the monoamine transporter. Besides, morphine interacts with α2 adrenergic receptors (α2A, α2B and α2C), on the contrary, fentanyl did not show affinity for α2 adrenoceptors, this effect may have an impact on the pharmacological actions of morphine. Also, it has been reported that various opioids inhibit monoamine transporters (NET and SERT) and interact with 5-HT receptors. Fentanyl has affinity for the 5-HT1A and 5-HT2A receptors, but there are no correlated data available regarding monoamine transporters, for both fentanyl and morphine (Sirohi et al. 2008; Hocker et al. 2009; Keith et al. 2019; Luethi and Liechti 2020).

The preceding antecedents show that there are interactions between opioids that are modulated by other non-opioid receptors in addition to the opioid receptors themselves and that could explain the differences between the antinociceptive activity induced by morphine and that obtained by fentanyl in the present work.

Conclusion

The present study, the efficacy of fentanyl in FHP and TF assays was relatively greater compared to the morphine. The greater effectiveness of fentanyl could lie by a different binding capacity or in the regulation or activation of opioids and non-opioid receptors. Co-administration of morphine with fentanyl induces synergism in all murine trials, confirming the antinociceptive capacity of both opioids, which would constitute a promisory inkling to treatment of pain.

Abbreviations

ED_{50}: dose that induce 50 & of MPE; MPE:maximun possible effect; MOR:receptor opioide mu; DOR:receptor opioide delta; KOR:receptor opioide kappa; NOP:receptor opioide nociceptina; GRKs:G protein receptor kinases; MAP:mitogen-activated kinase; TF:tail flick test; FHP:formalin hind paw test; I.I.:interaction index.

Declarations

Compliance with ethical standards

Ethical approval: All procedures with animals were approved by the Animal Care and Use Committee of the Faculty of Medicine of Universidad de Chile. (Protocol CBA 0852/FMUCH/2018).

Competing interests

All the authors declare that they have no conflicts of interest

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Not applicable

Consent to Participate and Consent to Publish

Informed consents were obtained from all the participant in this study

Authors Contributions

HFM and VN conceived and designed research and wrote the manuscript. VN and FS performing experiments. RSZ and JCP analyzed and cooperate in the interpretion of data. All authors read and approved the manuscript and submission. All data were generated in-house and that no paper mill was used.

References

Ananthan S. (2006) Opioid Ligands with Mixed / Opioid Receptor Interactions: An Emerging Approach to Novel Analgesics AAPSJ 8: E118-E125.

Corder G, Castro DC, Bruchas MR, et al (2018) Endogenous and Exogenous Opioids in Pain. Annu Rev Neurosci 41: 453 -473.

Höcker J, Böhm R, Meybohm P, et al. (2009) Interaction of Morphine but Not Fentanyl with Cerebral alpha2-adrenoceptors in alpha2-adrenoceptor Knockout Mice. J Pharm Pharmacol 61: 901-910.

Keith M OlsonKM, Duron D, et al. (2019) Comprehensive Molecular Pharmacology Screening Reveals Potential New Receptor Interactions for Clinically Relevant Opioids. doi:10.1371/ Plos one 14 (6) e 0217371.

Lemel L, Lane JR, Canals M (2020) GRKs as Key Modulators of Opioid Receptor function. Cells 9 (11), 2400;doi:10.3390/cells9112400.

Luethi D, Liechti ME. (2020) Designer drugs: mechanism of action and adverse effects. Archives of Toxicology 94:1085–1133

Miranda HF, Noriega V, Zepeda RJ, et al. (2012) Synergism between fentanyl and tramadol in tonic inflammatory pain: the orofacial formalin test. Inflammation 35: 1132-1137

Miranda HF, Puig MM, Dursteler C, et al. (2007) Dexketoprofen-induced antinociception in animal models of acute pain: synergy with morphine and paracetamol. Neuropharmacology 52: 291-296.

Miranda HF, Sierralta F, Pinardi G (2001) An isobolographic analysis of the adrenergic modulation of diclofenac antinociception. Anesth Anal 93(2): 430-435.

Morgan MM, Tran A, Wescom RL, Bobeck EN. (2020) Differences in antinociceptive signalling mechanisms following morphine and fentanyl microinjections into the rat periaqueductal gray. Eur J Pain

24 (3): 617- 6324.

Pasternak GW. (2004) Multiple opioid receptors: deja vu again. Neuropharmacology 47 Suppl 1: 312–323.

Pathan H, Williams J (2012) Basic opioid pharmacology: an update Br J Pain 2012; 6: 11–16.

Romero A, Miranda HF, Puig MM. (2010) Antinociceptive effects of morphine, fentanyl, tramadol and their combination, in morphine-tolerant mice. Pharmacol Biochem Behav 97: 363-369.

Sirohi S, Dighe S V, Walker EA, et al. (2008) The analgesic efficacy of fentanyl: Relation to tolerance and μ- opioid receptor regulation. Pharmacol Biochem Behav 91: 115-120.

Tallarida RJ. Drug synergism: its detection and applications. (2001) J Pharmacol Exper Ther 298: 865 – 872.

Waldhoer M, Barlett SE, Whisther J. (2004) Opioid receptor. Annu Rev Biochem 73: 953-990.

Zaveri N, Polgar WE, Olsen CM, et al. (2001)Characterization of opiates, neuroleptics, and synthetic analogs at ORL1 and opioid receptors. Eur J Pharmacol 428: 29-36.

Tables

TABLE 1: ED_{50} values with SEM in mg/kg and analgesic ratio (AR) for the antinociceptive activity of morphine and fentanyl in algesimeter tests of mice.

TEST	ED_{50} ± SEM		AR^a	AR^b	AR^c
	MORPHINE	FENTANYL			
FHP-I	0.17 ± 0.02	0.055 ± 0.003	29.47	1.25	3.09
FHP-II	0.38 ± 0.03	0.033 ± 0.001	13.18	2.09	11.52
TF	5.01 ± 0.91	0.069 ± 0.002	1.00	1.00	29.84

FHP-I: formalin hind paw phase I, FHP-II: formalin hind paw phase II, TF: tail flick. [a] compared with morphine in TF, [b] compared with fentanyl in TF, [c] comparison between fentanyl with morphine

TABLE 2: Theoretical and experimental ED_{50} values with SEM in mg/kg and interaction index (I.I.) values for the antinociceptive interaction of morphine with fentanyl (M/F) in algesimeter tests of mice.

M/F	ED50 ± SEM		I.I.	Interaction
	THEORETICAL	EXPERIMENTAL		
TF	2.53 ± 0.05	1.03 ± 0.02	0.406	Synergistic
FHP-I	0.11 ± 0.01	0.025 ± 0.002	0.223	Synergistic
FHP-II	0.20 ± 0.01	0.035 ± 0.008	0.169	Synergistic

TF: tail flick, FHP-II: formalin hind paw, phase II, , FHP-1: formalin hind paw, phase I

Figures

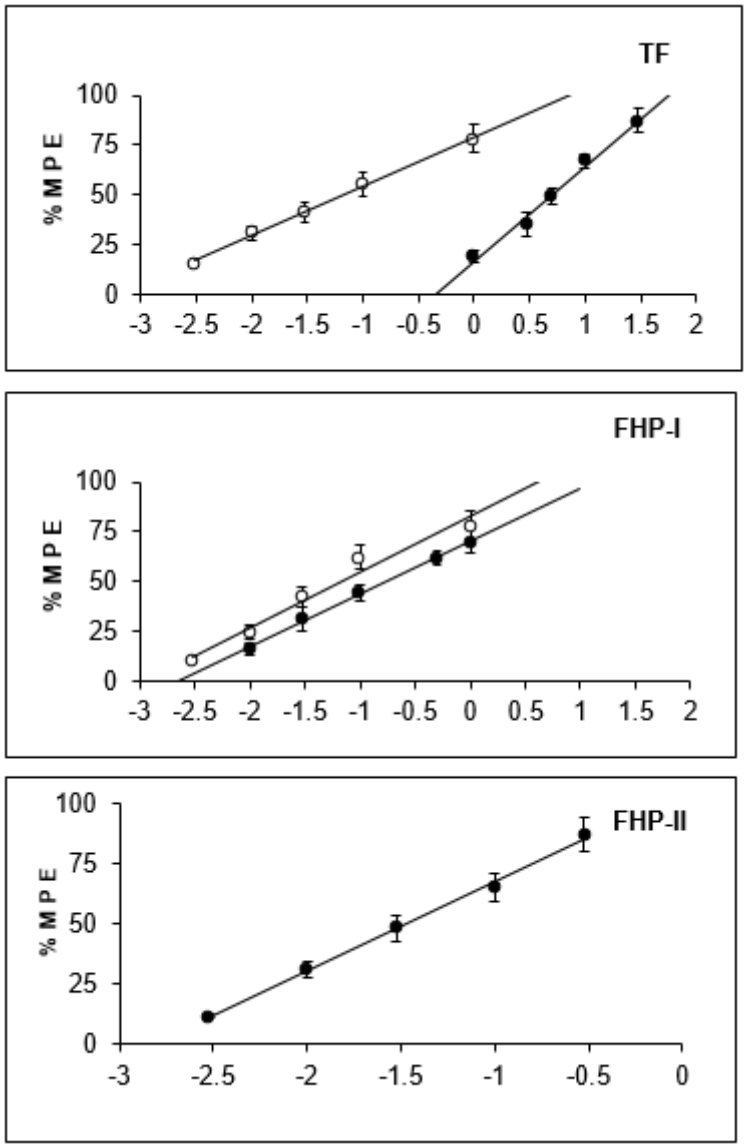

Figure 1

Dose-response for the antinociceptive activity induced by morphine (●) and fentanyl (⬤) in the formalin hind paw (FHP), phase I and II and tail flick (TF) assays of mice. Each point is the mean of 6-8 mice. % MPE represent antinociception evaluated as percentage of maximum possible effect. Abscissa is log of dose of fentanyl or morphine.

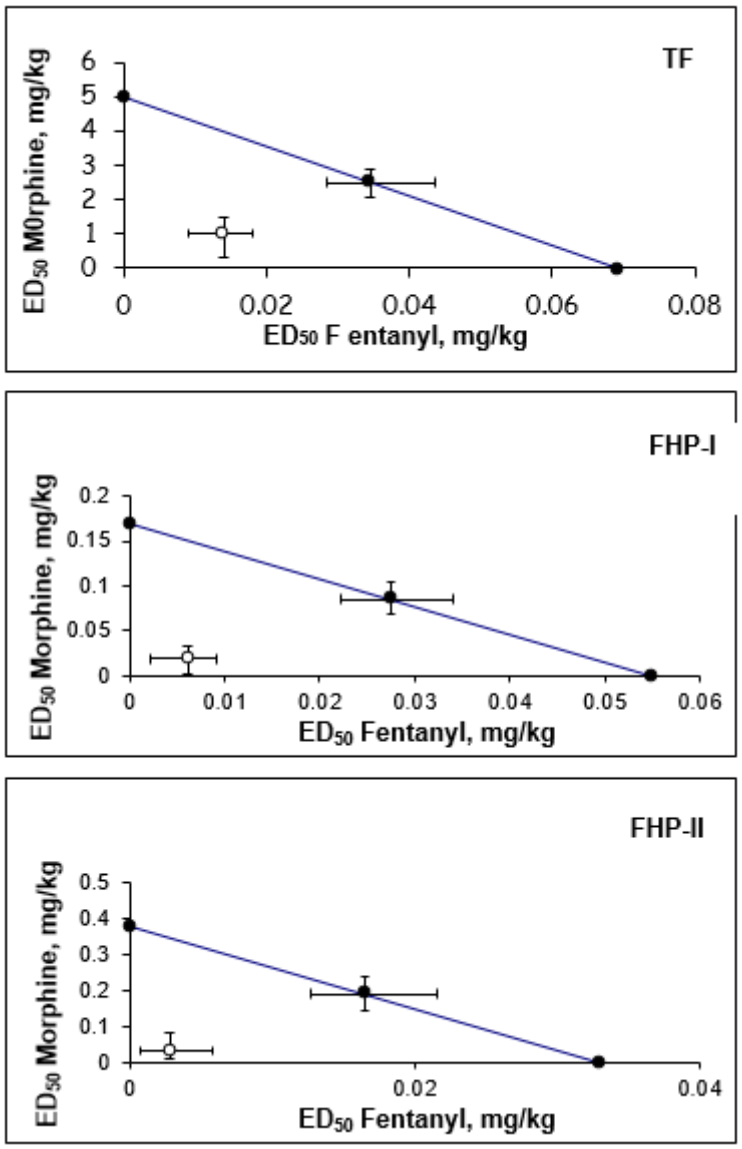

Figure 2

Isobolographic representation of the antinociceptive activity of the i.p. coadministration of fentanyl with morphine in the tail flick (TF) and formalin hind paw (FHP) phase I and II assay of mice. (●).

Supplementary Files

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