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Evaluation of the Centrally-Acting Mechanisms of Some Non-Steroidal Anti-inflammatory Drugs

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ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) whose main mechanism is inhibition of cyclooxygenases are commonly used for the management of pain. However, this mechanism is inadequate to explain the central analgesic activity of these drugs. We aimed to examine and to compare the central analgesic activity of diclofenac, etodolac, and indomethacin and the possible involvement of serotonergic and adrenergic mechanisms in their effects by using the hot plate and tail immersion tests. All drugs were assessed at same single doses (10 mg/kg). Mice were pretreated with prazosin and yohimbine, adrenergic antagonists, (1 mg/kg; i.p., 30 min. before testing) for investigating adrenergic mechanisms. Ondansetron and ketanserin, serotonergic antagonists, (0.5 mg/kg; i.p., 30 min. before testing) were also used for investigating serotonergic mechanisms. In tail immersion test, yohimbine and ketanserin decreased the antinociceptive effects of all tested NSAIDs. Ondansetron only antagonized the antinociceptive effect of diclofenac while prazosin was found ineffective. In the hot plate test, prazosin attenuated the antinociceptive effect of indomethacin and etodolac. Yohimbine, ondansetron, and ketanserin reversed the antinociceptive effects of all test drugs. The antinociception induced by tested drugs appears to be mediated either serotonergic (5-HT_2/5-HT_3) or adrenergic (α_1/α_2) receptors in spinal/supraspinal level. 5-HT_2/ 5-HT_3 and α_1/α_2 receptors are more associated with the supraspinal antinociceptive effect of NSAIDs while 5–HT_2 and α_2 receptors are predominantly involved in antinociceptive effect at the spinal level.

Keywords: Diclofenac; etodolac; indomethacin; pain; central mechanisms.

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INTRODUCTION

Pain is described as an unpleasant sensory and emotional experience arising from any part of the body and associated with actual and potential tissue damage or described in terms of such damage by international association for the study of pain-IASP.[1] Non-steroidal anti-inflammatory drugs (NSAIDs) are used for the management of pain, fever and inflammation for a long time. It is known that antinociceptive effects of NSAIDs are due to suppression of prostaglandin synthesis activity through cyclooxygenase (COX) inhibition in the periphery [2, 3]. It is also well known that NSAIDs block synthesis of prostaglandin via COX inhibition in the central nervous system as well and elicit central antinociception by this mechanism and some others[4, 5]. However, it is inadequate to explain the central mechanism of action of these drugs. Besides Cashman[6] expressed that interference with G-protein-mediated signal transduction by NSAIDs may form the basis of an analgesic mechanism unrelated to inhibition of prostaglandin synthesis. Some studies focused on to achieve additional centrally mediated mechanisms at supraspinal or spinal levels[6, 7, 8]. It is known that there are many neurotransmitters in pain processing and modulation in central nervous system. Among of these neurotransmitters serotonin (5-hydroxytryptamine / 5-HT) and noradrenaline, especially involve in descending inhibitory pathways, are the most impressive modulators.[9] 5-HT_1, 5-HT_2 and 5-HT_3 receptors are involved in the 5-HT mediated antinociceptive mechanism as well as α_1 and mostly α_2 adrenergic receptors involved in noradrenaline-mediated antinociception[10, 11, 12]. Tail immersion and hot plate tests are commonly used methods that assess the thermal nociceptive threshold. Both two tests are characterized by their tendency to respond to the noxious stimuli conducting through neuronal pathways, as the tail immersion mediates spinal reflexes to nociceptive stimuli while the hot plate mediates supraspinal response of pain[13, 14, 15]. As mentioned before, NSAIDs inhibit the COX and decrease peripheral and central prostaglandin production. However, the other centrally-acting mechanisms of NSAIDs are not adequately identified. In the present study, performed by using the hot plate and tail immersion tests, we aimed to examine the central analgesic activity of diclofenac (inhibits both COX-1 and COX-2) [16,17], etodolac (inhibits COX-2 more selective than COX-1)[13,14] and indomethacin (inhibits both COX-1 and COX-2)[13,14] and compare the role of serotonergic and adrenergic mechanisms in their effects.

MATERIALS AND METHODS

Drugs and chemicals

Diclofenac, etodolac and indomethacin were tested in this study and provided by the following

sources: etodolac (Bilim Pharmaceuticals, Istanbul, Turkey), diclofenac sodium, indomethacin, prazosin HCl and yohimbine HCl; adrenergic antagonists, ondansetron HCl and ketanserin tartrate; serotonergic antagonists, were purchased by Sigma, St. Louis, USA. 20 % DMSO (80 mL saline water, 20 mL DMSO) (Merck, Darmstadt, Germany) used as the vehicle.

Animals

Balb-c mice (30-40 g) were gotten from Anadolu University Experimental Animals Research Centre, Eskisehir, Turkey. Animals were housed in a laboratory with controlled temperature (22 ± 2°C) for 12 h light/12 h dark cycle with free access to food and water. Animal care and research protocols were based on the principles and guidelines adopted by the Guide for the Care and Use of Laboratory Animals (NIH publication No: 85-23, revised in 1985) and approved by the Local Ethics Committee of Anadolu University, Eskisehir, Turkey.

Drugs and administration

All drugs (diclofenac, etodolac, and indomethacin) were administered same single doses (10mg/kg) by intraperitoneal (i.p.) route. Mice pretreated with prazosin and yohimbine (1 mg/kg; *i.p.*, 30 min. before drug administration), ondansetron and ketanserin, (0.5 mg/kg; *i.p.*, 30 min. before drug administration) were also tested. The vehicle was injected to the control groups at the same volume (0.1 mL).

Tail immersion test

The tail immersion test was performed to assess the response to thermal stimuli.[18] The tip of the tail of mice were dipped in hot water at 52.5 ± 0.2°C (Heto, Allerod, Denmark) (n= 8-9). The latency of the tail withdrawal from hot water (in seconds) was measured 30 min after treatment and recorded as reaction time. To avoid tissue damage, a maximum latency of 15 s was applied.

Hot plate test

Pain reflexes in response to a thermal stimulus were evaluated using a Hot Plate Analgesia Meter (Ugo Basile Instruments; No.7280) as previously reported by Eddy and Leimbach at 1953.[19] Pain response was measured 30 min after drug injection. The mice (n = 8-9) were put on the plate (55° ± 0.5°C) after and the latencies for paw licking, hindpaw flicking or jumping were recorded for each animal. The cut off time was 20 s for to avoid any paw damage.

Statistical Analysis

The statistical analyzes were carried out using GraphPad Prism version 5.0. Data obtained from animal experiments were expressed mean values ± standard error of mean (S.E.M.). Statistical differences were calculated by one-way ANOVA, followed by Tukey's multiple comparison tests. The results were expressed as the differences were considered significant when $P \leq 0.05$.

The results of the tail immersion and hot plate tests were given as a percentage of the maximal possible effect (MPE%±S.E.M.), which was calculated as follows:[20]

MPE% = [(postdrug latency)-(predrug latency) / (cutoff time)-(predrug latency)] x 100

RESULTS AND DISCUSSIONS

In the present study, it was found that intraperitoneal administration of several NSAIDs showed significantly antinociceptive activities in the tail immersion and hot plate tests. Additionally, it was investigated that the adrenergic and serotonergic mechanisms involved in these antinociceptive effects. NSAIDs have anti-inflammatory, analgesic and antipyretic effects, and they primarily inhibit the synthesis of prostaglandins. However, but this is not completely clarify the antinociceptive effect of NSAIDs and needs to be elucidated[7]. Epidemiological studies indicate that NSAIDs are the neuroprotective effect in Alzheimer's disease (AD) and other neurodegenerative disorders. Some researchers investigated that NSAIDs permeated across blood-brain barrier in vitro and in vivo studies[21; 22; 23]. Diclofenac has anti-inflammatory, analgesic, and antipyretic effects. It inhibits the COX enzymes (nonselective COX-1 and COX-2) and reduces the production of prostaglandins[24; 25]. Etodolac is one of NSAIDs, which is more selective for the COX-2 enzyme than COX-1. Indomethacin is another NSAID, the nonselective COX inhibitor, and mostly used for the treatment of rheumatoid diseases.[26; 27] In our study, we evaluated the contribution of central serotonergic and noradrenergic pathways on the antinociceptive mechanism of diclofenac, etodolac and indomethacin by using tail immersion and hot plate tests, acute thermal pain models. According to tail immersion test results; diclofenac ($p<0.01$), etodolac and indomethacin ($p<0.001$) showed significantly antinociceptive activities in tail immersion test when compared to control group (Figure 1). Prazosin did not show an antagonistic effect on using NSAIDs. However, the antinociceptive effect of diclofenac, etodolac, and indomethacin were antagonized by 1 mg/kg yohimbine ($p<0.05$, $p<0.01$, $p<0.001$: respectively) (Figure 2). Ondansetron just affected on diclofenac significantly ($p<0.001$). Ondansetron also antagonized the antinociceptive effect of indomethacin and etodolac, but this antagonism was relative. Diclofenac and indomethacin ($p<0.001$), etodolac ($p<0.05$) were antagonized by ketanserin (Figure 3).

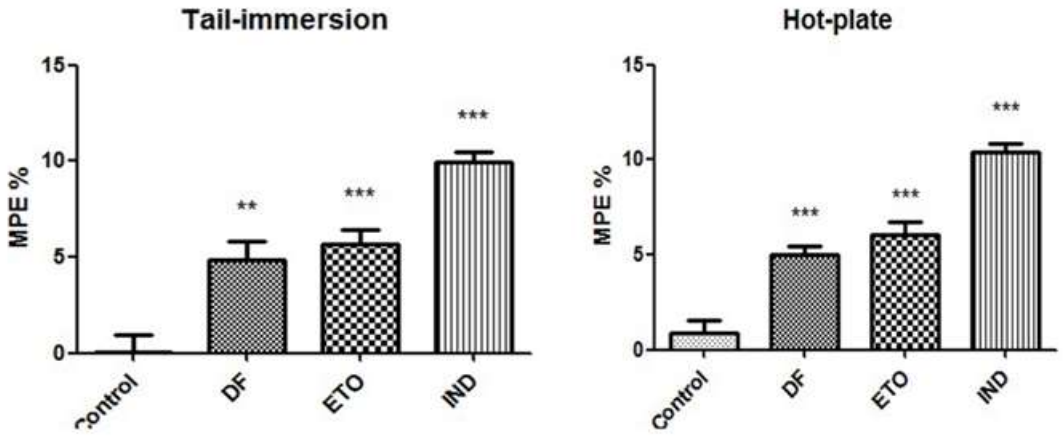

Figure 1: The antinociceptive activities of NSAIDs in tail immersion and hot plate test, compared to control group ($p<0.01$; *** $p<0.001$). Values are presented as the mean±S.E.M. DF: diclofenac, ETO: etodolac, IND: indomethacin.**

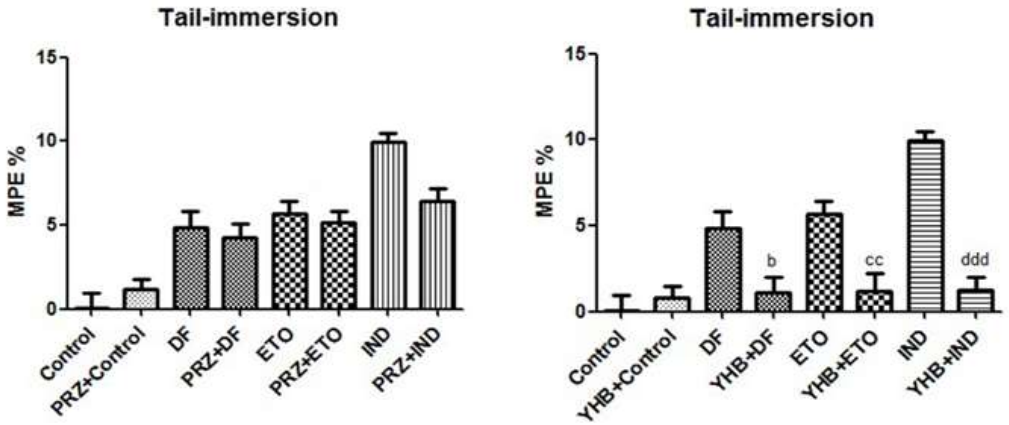

Figure 2: Antagonistic effects of prazosin and yohimbine in tail immersion test, the significant difference from diclofenac ($^{b}p<0.05$), etodolac ($^{cc}p<0.01$), indomethacin ($^{ddd}p<0.001$) alone. Values are presented as the mean±S.E.M. DF: diclofenac, ETO: etodolac, IND: indomethacin, PRZ: prazosin, YHB: yohimbine.

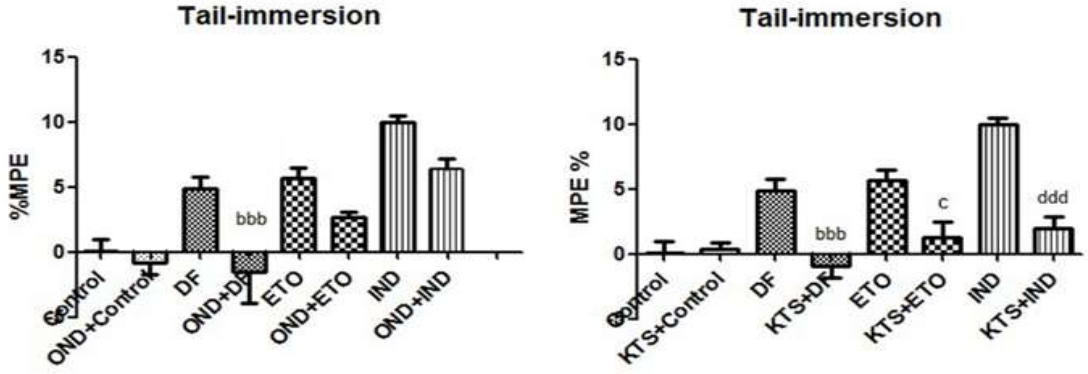

Figure 3: Antagonistic effects of ondansetron and ketanserin in tail-immersion test, the significant difference from diclofenac ($^{bbb}p<0.001$), etodolac ($^{c}p<0.05$), indomethacin ($^{ddd}p<0.001$) alone. Values are presented as the mean±S.E.M. DF: diclofenac, ETO: etodolac, IND: indomethacin, OND: ondansetron, KTS: ketanserin

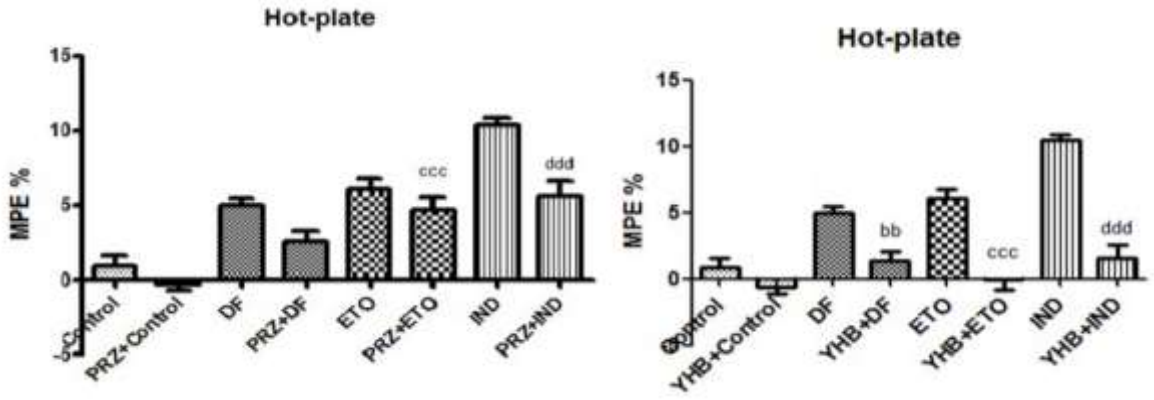

Figure 4: Antagonistic effects of prazosin and yohimbine in the hot plate test, the significant difference from diclofenac ($^{bb}p<0.01$), etodolac ($^{ccc}p<0.001$), indomethacin ($^{ddd}p<0.001$) alone. Values are presented as the mean±S.E.M. DF: diclofenac, ETO: etodolac, IND: indomethacin, PRZ: prazosin, YHB: yohimbine.

The antinociceptive activities of NSAIDs in the hot plate test are shown in Fig 1. It was demonstrated that all tested NSAIDs possess significant antinociceptive activities when compared to control group ($p<0.001$). The reversal effects of adrenergic and serotonergic antagonists on the antinociceptive activities of NSAIDs were demonstrated in Fig 4 and 5. Prazosin (1mg/kg) significantly antagonized the antinociceptive effect of indomethacin and etodolac ($p<0.001$). Administration of yohimbine antagonized the antinociceptive effect of NSAIDs in the hot plate test (diclofenac $p<0.01$; etodolac and indomethacin $p<0.001$). Ondansetron counteracted antinociceptive activity of NSAIDs (diclofenac $p<0.001$; etodolac $p<0.01$ and indomethacin $p<0.001$). Ketanserin, significantly antagonized the effect of all tested NSAIDs ($p<0.001$) in the hot plate test.

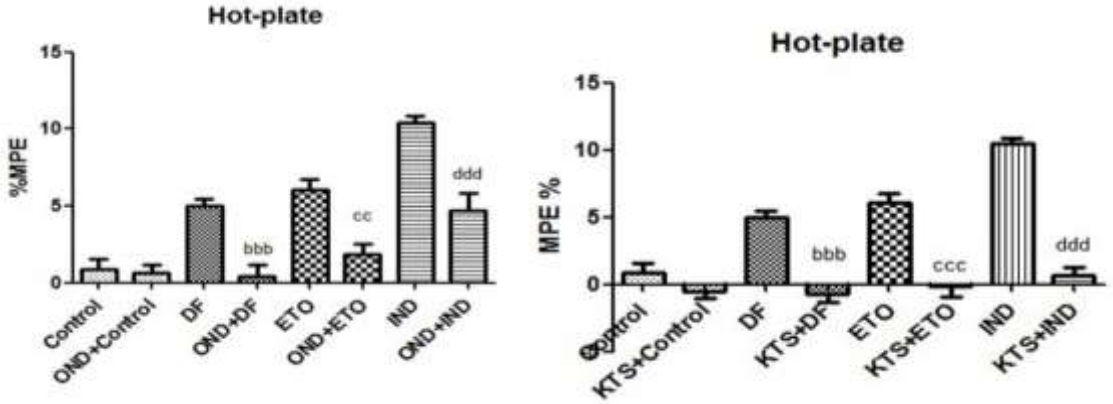

Figure 5: Antagonistic effects of ondansetron and ketanserin in hot-plate test, significant difference from diclofenac ($^{bbb}p<0.001$), etodolac ($^{cc}p<0.01$; $^{ccc}p<0.001$), indomethacin ($^{ddd}p<0.001$) alone. Values are presented as the mean±S.E.M. DF: diclofenac, ETO: etodolac, IND: indomethacin, OND: ondansetron, KTS: ketanserin.

Diclofenac, etodolac, and indomethacin elevated the thermal threshold in this two model and found significantly effective when administered at same doses. Thereby, it was thought that tested NSAIDs exhibit the antinociception both at the spinal and supraspinal levels. It was also observed that indomethacin was more potent than others. There are other studies compatible with our results that reveal the central antinociceptive effects of NSAIDs. For instance, in a study ketoprofen, diclofenac and piroxicam showed the antinociceptive effect in the tail-flick test, another test model that evaluates central antinociception.[5] In another study, the antinociceptive activity of paracetamol was found significantly effective both in the first phase of formalin test and hot-plate test.[28] It is known that the formalin test involves two distinctive phases, first and second phases, and centrally acting drugs are effective in both phases. It is reported that the main mechanism for antinociception induced by NSAIDs is inhibition of COX enzymes. It is also known that additional mechanisms are involved in the central antinociceptive effect of NSAIDs besides the contribution of COX inhibition. Thereby, recent studies focus on this topic.[4; 7; 29] The central action mechanisms of NSAIDs were searched by some investigators. According to results of study performed by Pinardi et al.[5], an activation of α_2-adrenoceptors located at spinal and supraspinal levels were contributed to antinociception induced by ketoprofen, diclofenac and piroxicam, whereas central activation of the descending noradrenergic inhibitory system by α_1-adrenoceptors was probably involved in antinociceptive effect of paracetamol[5], as similarly our results. The involvement of 5-HT in the antinociception induced by some NSAIDs investigated in the mouse writhing test, used for detecting both central and peripheral analgesia, and the tail-flick test, after the inhibition of 5-HT biosynthesis by administration of p-chlorophenylalanine (i.p.). It has been suggested that the activation of descending serotonergic pathways is the supplementary mechanism of antinociception for NSAIDs.[7] It is also thought that the antinociceptive effect of NSAIDs may depend on the presence of muscarinic cholinergic sites that harmonize the transmission of afferent nociceptive stimuli when injected intrathecal (i.t., spinal administration) and intraperitoneal (systemic administration) route. It has been concluded that pre- and postsynaptic mechanisms that facilitate cholinergic transmission are related to antinociception of NSAIDs since atropine or HC-3 antagonized the antinociceptive effect of NSAIDs in acute thermal pain models[29]. It is known that NSAIDs enhanced the effectiveness of other antinociceptive agents. To enhance analgesia, the strategy that offers the combined use of analgesic drugs with proven efficacies to attenuate adverse reactions is a commonly-used.[30; 31] The systemic administration of morphine and NSAIDs (diclofenac, ketoprofen, meloxicam, metamizole, paracetamol, and piroxicam) induced antinociception in a dose-dependent manner in

the mouse writhing test. The isobolographic analysis performed when used in combination with the simultaneous i.p. injection of fractions of morphine and each NSAID showed the presence of a supra-additive interaction. In this study, naltrexone, μ-opioid receptors antagonist, naltrindole, δ-opioid receptor antagonist, and nor-binaltorphimine, κ-opioid receptor antagonist, were used to assess the mechanism of synergism. The data obtained from this experiments demonstrated that the synergistic interaction between morphine and NSAIDs might be probably associated with the different intracellular signal transduction mechanisms involved in the effect of opioid and non-opioid drugs[31]. In another study, the interactions between intrathecal NSAIDs (naproxen, piroxicam, paracetamol, dipyrone or metamizole and nimesulide) and the α_2-adrenoceptor agonist clonidine (i.p.) was evaluated in the writhing test. It has been suggested that the synergistic interactions between systemic NSAIDs and clonidine could involve supraspinal mechanisms[32]. Also Miranda and Pinardi[33], studied the (i.t) combination of opioids and NSAIDs in the writhing test. The supra-additive interaction was obtained with NSAIDs (diclofenac, ketoprofen, meloxicam, metamizole, naproxen, nimesulide, parecoxib and piroxicam) when administered at very low doses. It was not significantly modified by i.t. naltrexone. Thereby, it has been thought that the usage of morphine and NSAIDs in combination has a direct action on spinal nociceptive processing, which may be mediated by mechanisms that are independent of the activation of opioid receptors. The spinal cord is the lowest level of the central nociceptive system and under the influence of descending tracts[34]. Descending pain modulation pathways are one of the most important pain regulatory pathways[35; 36]. These pathways are the neuronal link between the cortex, hypothalamus, and amygdala to control ascending pain at the level of the reticular formation, midbrain areas, and the spinal cord. The action and releasing of various spinal neurotransmitters as endogenous opioids, noradrenaline, serotonin, and acetylcholine are contributed to the mechanism of descending inhibition and supraspinal/spinal signal integration[37]. Thereby, adrenergic and serotonergic mechanisms play a key role in the modulation of nociception transmission[5; 11; 38; 39; 40]. The studies reported that noradrenaline produces analgesia directly via presynaptic or postsynaptic α_2-adrenergic receptors and indirectly α_1-adrenergic receptors[41]. The effect of serotonin on pain may be inhibitor or facilitator characteristic according to receptor location. However, it is known that 5-$HT_{2A/C}$ and 5-HT_3 receptors play the role in serotonergic antinociception[42]. In this study, in order to evaluate the antinociceptive mechanisms in central nervous system of selected NSAIDs, prazosin (selective α_1-adrenoceptor antagonist) and yohimbine (nonselective α_2-adrenoceptor antagonist); ketanserin (5-$HT_{2A/C}$ receptor antagonist) and ondansetron (5-HT_3 receptor antagonist) were used for

adrenergic and serotonergic pathway respectively. In order to investigate the centrally mediated antinociception both hot plate and tail immersion tests are used. They are categorized by the respond to the noxious stimuli conducting through neuronal pathways, as the tail immersion mediates spinal reflexes to nociceptive stimuli while the hot plate mediates supraspinal response of pain[15; 17]. Yohimbine antagonized the effect of selected NSAIDs both in the tail immersion and hot plate tests while prazosin antagonized the antinociceptive effect of indomethacin and etodolac in the hot plate test. Hayes et al.[43] observed that antinociception may be mediated by either α_1 or α_2-adrenoceptors and these subtypes of adrenoceptors found in spinal and supraspinal regions[9; 43; 44]. Our results demonstrated that α_1-adrenoceptors involved in antinociceptive effect of indomethacin and etodolac at only supraspinal level, because of thermal threshold decreased by prazosin pretreatment in hot-plate test, although α_2-adrenoceptors involved in antinociception of each NSAID at spinal and supraspinal level. Moreover, the analgesic effect of etodolac and indomethacin could not be antagonized with ondansetron while ketanserin blocked the antinociceptive effect of all NSAIDs in tail immersion tests. Interestingly, 5-HT_3 receptor antagonist ondansetron achieved to reverse the antinociceptive effect of diclofenac. However, the role of 5-HT_3 receptors in pain modulation is controversial, therefore, needs further investigation about the 5-HT_3 associated mechanism of diclofenac[10; 45]. Additionally, ketanserin and ondansetron both antagonized the antinociceptive effect of all selected NSAIDs in hot-plate tests. Thereby, it can be concluded as, both 5-$HT_{2A/C}$ and 5-HT_3 serotonin receptors at supraspinal level play an important role in the antinociceptive effect of NSAIDs.

CONCLUSION

Each NSAID, used in this study, showed centrally mediated antinociception at both spinal and supraspinal levels. The antinociceptive effect of NSAIDs appears to be mediated by either serotonergic (5-$HT_{2A/C}$/5-HT_3) or adrenergic (α_1/α_2) receptors in spinal/supraspinal level. 5-$HT_{2A/C}$, 5-HT_3 receptors, and α_2 adrenoceptor subtypes are more associated with the supraspinal antinociceptive effect of NSAIDs while 5-$HT_{2A/C}$ serotonin and α_2 adrenoceptor subtypes are predominantly involved in spinal antinociception induced by NSAIDs.

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REFERENCES

1. Merskey H, Bogduk N. Part III: pain terms, a current list with definitions and notes on usage, in: Merskey H., Bogduk N. (Eds.), Classification of Chronic Pain, IASP Task Force on

Taxonomy. 2nd ed. IASP Press, Seattle: WA, pp. 1994;209-214.

2. Sawynok J. Topical and Peripherally Acting Analgesics. Pharmacol Rev, 2003;55:1-20.
3. Day RO, Graham GG. Non-steroidal anti-inflammatory drugs (NSAIDs). BMJ 2013;346:f 3195.
4. Vanegas H, Schaible HG. Prostaglandins and cycloxygenases in the spinal cord. Prog Neurobiol 2001;64:327–363.
5. Pinardi G, Sierralta F, Miranda HF. Adrenergic mechanisms in antinociceptive effects of nonsteroidal anti-inflammatory drugs in acute thermal nociception in mice. Inflamm Res 2002;51:219–222.
6. Cashman JN. The mechanisms of action of NSAIDs in analgesia. Drugs 1996;52 Suppl 5:13-23.
7. Miranda HF, Lemus I, Pinardi G. Effect of the inhibition of serotonin biosynthesis on the antinociception induced by nonsteroidal anti-inflammatory drugs. Brain Res. Bull 2003;61:417-425.
8. Sandrini M, Pini LA, Vitale G. Differential involvement of central 5-HT_{1B} and 5-HT_3 receptor subtypes in the antinociceptive effect of paracetamol. Inflamm Res 2003;52:347-352.
9. Yokogawa F, Kiuchi Y, Ishikawa Y, Otsuka N, Masuda Y, Oguchi K, Hosoyamada A (2002). An Investigation of Monoamine Receptors Involved in Antinociceptive Effects of Antidepressants. Anesth Analg., 95:163–168
10. Sasaki M, Ishizaki K, Obata H, Goto F. Effect of 5-HT_2 and 5-HT_3 receptors on the modulation of nociceptive transmission in rat spinal cord according to the formalin test. Eur J Pharmacol 2001;424:45-52.
11. Dogrul A, Coskun I, Uzbay T. The contribution of alpha-1 and alpha-2 adrenoceptors in peripheral imidazoline and adrenoceptor agonist-induced nociception. Anesth. Analg 2006;103:471-77.
12. Silveira JW, Dias QM, Del Bel EA, Prado, WA. Serotonin receptors are involved in the spinal mediation of descending facilitation of surgical incision-induced increase of Fos-like immunoreactivity in rats. Mol. Pain, 2010;6:17.
13. Chapman CR, Casey KL, Dubner R, Foley KM, Gracely RH, Reading AE. Pain measurement: an overview. Pain 1985;22(1):1-31.
14. Morales L, Perez-Garcia C, Alguacil LF. Effects of yohimbine on the antinociceptive and place conditioning effects of opioid agonists in rodents. Br J Pharmacol 2001;133(1):172-8.

15. 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(1-2):205-14.
16. Rao P, Knaus EE. Evolution of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs): Cyclooxygenase (COX) Inhibition and Beyond. J Pharm Pharmaceut Sci 2008;11(2):81s-110s.
17. Brune K, Patrignani P. New insights into the use of currently available non-steroidal anti-inflammatory drugs. J Pain Res 2015;8:105-18
18. Schmauss C and Yaksh TL. In vivo studies on spinal opiate receptor systems mediating antinociception. II. Pharmacological profiles suggesting a differential association of mu, delta and kappa receptors with visceral chemical and cutaneous thermal stimuli in the rat. J Pharmacol Exp Ther 1984;228:1-12.
19. Eddy NB, Leimbach D. Synthetic analgesics: II. Dithienylbutenyland dithienylbutylamines. J. Pharmacol. Exp. Ther 1953;107:385–393.
20. Coelho LP, Reis PA, Castro FL, da Silva Lopes C, da Costa e Silva MC, de Carvalho Sabino KC, Todeschini AR, Coelho MG. Antinociceptive properties of ethanolic extract and fractions of Pterodon pubescens Benth. seeds. J. Ethnopharmacol 2005;98:109–116.
21. Townsend KP, Pratico D. Novel therapeutic opportunities for Alzheimer's disease: focus on nonsteroidal anti-inflammatory drugs. FASEB J 2005;19:1592–1601.
22. Parepally JM, Mandula H, Smith QR. Brain uptake of nonsteroidal antiinflammatory drugs: ibuprofen, flurbiprofen, and indomethacin. Pharm Res 2006;23: 873–881
23. Novakova I, Subileau EA, Toegel S, Gruber D, Lachmann B, Urban E, Chesne C, Noe CR, Neuhaus W. Transport rankings of non-steroidal antiinflammatory drugs across blood-brain barrier in vitro models. PLoS One 2014;9(1):1-14.
24. Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ, Vane JR. Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. Proc. Natl. Acad. Sci. USA 1993;90(24):11693–11697.
25. Van Hecken A, Schwartz JI, Depré M, De Lepeleire I, Dallob A, Tanaka W, Wynants K, Buntinx A, Arnout J, Wong PH, Ebel DL, Gertz BJ, De Schepper PJ. Comparative inhibitory activity of rofecoxib, meloxicam, diclofenac, ibuprofen, and naproxen on COX-2 versus COX-1 in healthy volunteers. J Clin. Pharmacol 2000;40:1109-20.
26. Burke A, Smyth EM, FitzGerald GA. Chapter 26: Analgesics-antipyretic and antiinflammatory agents, in: Brunton L, Chabner B, Knollman B editors. Goodman &

Gilman's The Pharmacological Basic of Therapeutics, McGraw-Hill: U.S.A, pp. 2006;671-717.

27. Manrique-Moreno M, Garidel P, Suwalsky M, Howe J, Brandenburg K. The membrane-activity of Ibuprofen, Diclofenac, and Naproxen: A physico-chemical study with lecithin phospholipids. Biochim. Biophys. Acta 2009;1788:1296-303.
28. Pini LA, Sandrini M, Vitale G. The antinociceptive action of paracetamol is associated with changes in the serotonergic system in the rat brain. Eur J Pharmacol 1996;308: 31-40.
29. Pinardi G, Sierralta F, Miranda HF. Atropine reverses the antinociception of nonsteroidal anti-inflammatory drugs in the tail-flick test of mice. Pharmacol Biochem Behav 2003;74:603-608.
30. Seideman P, Melander A. Equianalgesic effects of paracetamol and indomethacin in rheumatoid arthritis. Br J Rheumatol 1988;27:117-22.
31. Miranda HF, Silva E, Pinardi G. Synergy between the antinociceptive effects of morphine and NSAIDs. Can J Physiol Pharmacol 200482(5):331-338.
32. Miranda HF, Pinardi G. Isobolographic analysis of the antinociceptive interactions of clonidine with nonsteroidal anti-inflammatory drugs. Pharmacol Res 2004;50:273-278.
33. Miranda HF, Pinardi G. Lack of effect of naltrexone on the spinal synergism between morphine and non-steroidal anti-inflammatory drugs. Pharmacol Rep 2009;61:268-274.
34. Schaible HG. Peripheral and central mechanisms of pain generation. Handb Exp Pharmacol 2007;177: 3-28.
35. Gebhart GF. Descending modulation of pain. Neurosci Biobehav Rev 2004;27:729-737.
36. Benarroch EE. Descending monoaminergic pain modulation: bidirectional control and clinical relevance. Neurology 2008;71:217-221.
37. Fiorino DF, Garcia-Guzman M. Muscarinic pain pharmacology: realizing the promise of novel analgesics by overcoming old challenges. Handb Exp Pharmacol 2012;208:191-221.
38. Bjorkman R. Central antinociceptive effects of non-steroidal anti-inflammatory drugs and paracetamol. Acta Anaesthesiol Scand 1995;Suppl 103:1-44.
39. Hunt TE, Wu WH, Zbuzek VK. Ondansetron blocks nifedipine analgesia induced in rats. Anesth Analg 1996;82:498-500.
40. Paul D, Yao D, Zhu P, Minor LD, Garcia MM. 5-Hydroxytryptamine3 (5-HT3) receptors mediate spinal 5-HT antinociception: an antisense approach. J Pharmacol Exp Ther 2001;298:674-678.

41. Ge ZJ, Zeng YM, Tan YF. Effects of intrathecal 6-hydroxydopamine, alpha1 and alpha2 adrenergic receptor antagonists on antinociception of propofol in mice. Acta Pharmacol Sin. 2005; 26(2):186-191.
42. Silveira JW, Dias QM, Del Bel EA, Prado WA. Serotonin receptors are involved in the spinal mediation of descending facilitation of surgical incision-induced increase of Fos-like immunoreactivity in rats. Mol Pain. 2010;6:17.
43. Hayes AG, Skingle M, Tyers MB. Antagonism of alpha-adrenoceptor agonist-induced antinociception in the rat. Neuropharmacology 1986; 25: 397-402.
44. Svokos K, Nalwalk JW, Leurs R, Menge WM, Timmerman H, Hough LB. A role for spinal, but not supraspinal, alpha (2) adrenergic receptors in the actions of improgan, a powerful, non-opioid analgesic. Brain Res 2001;923(1-2):12-19.
45. Zeitz KP, Guy N, Malmberg AB, Dirajlal S, Martin WJ, Sun L, Bonhaus DW, Stucky CL, Julius D, Basbaum AI. The 5-HT_3 subtype of serotonin receptor contributes to nociceptive processing via a novel subset of myelinated and unmyelinated nociceptors. J Neurosci 2002;22:1010-1019.