Acta Neuropsychiatrica 2015
All rights reserved
DOI: 10.1017/neu.2014.38

© Scandinavian College of Neuropsychopharmacology 2014
ACTA NEUROPSYCHIATRICA

Contribution of nociceptin/orphanin FQ receptors to the anti-nociceptive and hypothermic effects of dipyrone

Ertin IH, Gunduz O, Ulugol A. Contribution of nociceptin/orphanin FQ receptors to the anti-nociceptive and hypothermic effects of dipyrone.

Ismet Hande Ertin, Ozgur Gunduz, Ahmet Ulugol

Department of Medical Pharmacology, Faculty of Medicine, Trakya University, Edirne, Turkey

Background: Dipyrone is one of the most commonly used non-opioid analgesic and antipyretic drug. Its anti-nociceptive and hypothermic effects have long been suspected to be centrally mediated. The involvement of the most recently discovered opioid peptide, nociceptin/orphanin FQ (N/OFQ), and its receptor (NOP) in pain transmission is controversial. It appears to be pro-nociceptive when administered supra-spinally, but exerts anti-nociceptive effects when injected spinally or systemically.
Objective: Investigation of the role of the N/OFQ system in paracetamol-induced anti-nociception and hypothermia led us to determine its role in the anti-nociceptive and hypothermic effects of dipyrone.
Material and Methods: Hot-plate and tail-flick tests were used to assess nociception, and a rectal thermometer was used to measure rectal temperature in mice.
Results: Mice injected with dipyrone (150, 300, 600 mg/kg, i.p.) displayed dose-related anti-nociception and hypothermia. The NOP receptor antagonist JTC-801 (3 mg/kg, i.p.), at a dose that exerted no effect when used alone, alleviated dipyrone-induced anti-nociception but did not reverse dipyrone-induced hypothermia.
Conclusion: We conclude that NOP receptors participate in the anti-nociceptive, but not in the hypothermic, effects of dipyrone.

Keywords: anti-nociception; dipyrone; hypothermia; JTC-801; N/OFQ

Prof. Ahmet Ulugol, Department of Medical Pharmacology, Faculty of Medicine, Trakya University, 22030-Edirne, Turkey.
Tel/Fax: +90 284 235 3925
E-mail: aulugol@trakya.edu.tr; aulugol@yahoo.com

Accepted for publication November 6, 2014

First published online December 3, 2014

Significant outcomes

- Mechanisms mediating dipyrone analgesia and hypothermia are not totally understood. The nociceptin/orphanin FQ system is implicated in many physiological processes, including nociception and thermoregulation.
- Antagonising N/OFQ peptide receptors attenuates dipyrone-induced anti-nociception, but does not reverse hypothermia.

Limitations

- The site of N/OFQ peptide (NOP) receptor antagonist application can differentially modulate the nociceptin/orphanin FQ system; NOP antagonist may also be tested intra-thecally and intra-cerebroventicularly.
- Motor activity may also be tested to observe whether NOP receptor antagonist elicits any effect on motor function.

https://doi.org/10.1017/neu.2014.38 Published online by Cambridge University Press

Introduction

Dipyrone (metamizol) is an analgesic antipyretic drug that is being used worldwide to treat mild-to-moderate pain and to reduce elevated body temperature. Differently from the classical non-steroidal anti-inflammatory drugs (NSAIDs), it exerts little anti-inflammatory but strong analgesic and hypothermic actions. These effects of dipyrone have long been suspected to be centrally mediated (1,2); however, despite intensive investigations, the precise mechanisms mediating dipyrone analgesia and hypothermia are not fully elucidated. The periaqueductal grey matter (PAG), rostral ventromedial medulla (RVM) and the spinal cord appear to be the central targets of dipyrone, even when administered systemically (3,4). Descending inhibitory pathways and endogenous opioids seem to play roles in dipyrone actions (2–5). Moreover, recent research indicates that endogenous cannabinoid system may also participate in some of the pharmacological effects of dipyrone (6–8).

The endogenous ligand nociceptin/orphanin FQ (N/OFQ) and its receptor (N/OFQ peptide receptor, NOP) comprise a peptide receptor system expressed in brain areas implicated in many physiological processes, including nociception and thermoregulation. Although contradictory findings are obtained from studies on the effects of NOP receptor system on modulation of pain response, it appears that N/OFQ more often than not possesses supra-spinal hyperalgesic rather than spinal analgesic effects (9,10). In case of thermoregulation, it is known that administering N/OFQ causes hypothermia, whereas NOP receptor knockout results in hyperthermia (11,12).

Aims of the study

Like dipyrone, evidence also suggests a central component to the mechanism of action of paracetamol (13,14). N/OFQ administration has been shown to prevent the anti-nociceptive action of paracetamol on the rat hot-plate test (15). On the other hand, NOP receptors have been indicated not to mediate paracetamol-induced hypothermia in rats (16). Taking into account the similarities between dipyrone and paracetamol, the purpose of this work was to determine whether NOP receptors are involved in the anti-nociceptive and hypothermic effect of dipyrone.

Material and methods

Animals and ethics

Adult male Balb/c albino mice (Center of the Laboratory Animals, Trakya University) weighing 20–30 g were used. Animals were housed in groups of 10, and were maintained under controlled light (12 : 12 h light : dark cycles) and temperature (21 ± 2°C) conditions with water and food available *ad libitum*. This study was conducted according to the guidelines of the Ethical Committee of the International Association for the Study of Pain after approval by the 'Animal Care Ethics Committee' at Trakya University.

Hot-plate test

A conventional hot-/cold-plate analgesia meter (Ugo Basile, Comerio, Italy) was used to perform the hot-plate test. In brief, the animals were placed on an electrically heated plate that was kept constant at a temperature of 55 ± 0.1°C. Response latencies either to jump or a hind-paw lick were measured using an electronic timer. A cut-off time of 25 s was set in order to minimise tissue damage. Test latencies were converted to the percentage of the maximal possible effect (%MPE) according to the following equation: %MPE = [(post-drug latency – baseline latency)/cut-off time – baseline latency] × 100.

Tail-flick test

To perform the tail-flick test, a standardised tail-flick apparatus (Commat, Ankara, Turkey) was used. In brief, radiant heat was focussed on the dorsal surface of the mouse tail, and response latencies to a tail-flick were recorded using an electronic timer. Baseline tail-flick latency for each mouse ranged from 2 to 3 s. A cut-off time of 10 s was set in order to minimise tissue damage. Test latencies were converted to the %MPE according to the following equation: %MPE = [(post-drug latency – baseline latency)/cut-off time – baseline latency] × 100.

Measurement of rectal temperature

To measure rectal temperature of mice to the nearest 0.1°C by means of an Ellab thermometer, a 2-mm-diameter probe was inserted 2.5 cm into the rectum of mice and left there until steady readings were achieved.

Study design and drugs

Animals were randomly divided into control and treatment groups and habituated to the environment 3 days before the testing period. Subsequently, the effects of different doses of dipyrone (150, 300, 600 mg/kg, i.p.) and JTC-801 (1, 3, 10 mg/kg, i.p.), a NOP receptor antagonist, on nociception and rectal temperature were tested. Next, by administering the ineffective dose of JTC-801 (3 mg/kg) 30 min before dipyrone, the effect of NOP receptor antagonism on

https://doi.org/10.1017/neu.2014.38 Published online by Cambridge University Press

the anti-nociceptive and hypothermic effects of dipyrone were observed. Different cohorts of animals were used for JTC-801 administrations. Hot-plate, tail-flick and hypothermia tests were performed in all animals and each of them were used only once. For standardisation, the tests were conducted in the same order, tail-flick being the first and hypothermia the last.

Drug doses and treatment times were selected from previous studies (17–19). Dipyrone was purchased from Santa Cruz, and JTC-801 from Sigma Chemical Co. Dipyrone was dissolved in saline and JTC-801 was dissolved in 20% DMSO, 5% Tween 80, 5% ethanol and 70% saline; both were administered i.p. in a volume of 0.1 ml/10 g body weight.

Statistical analysis

Analysis of variance followed by the Bonferroni t test were used to compare the data of the groups. Values of $p < 0.05$ were considered to be statistically significant. All data are expressed as mean ± SEM.

Results

Dipyrone (150–600 mg/kg, i.p.) administration exerted dose-dependent anti-nociceptive and hypothermic effects (Fig. 1). The NOP receptor antagonist, JTC-801 (1, 3, 10 mg/kg, i.p.), did not affect either nociception or body temperature (data not shown). JTC-801 (3 mg/kg), at a dose that elicited no action on its own, partially abolished the anti-nociceptive action of dipyrone in both hot-plate and tail-flick tests, but did not reverse dipyrone-induced hypothermia (Fig. 2). JTC-801 augmented the hypothermic effect of 300 mg/kg dose of dipyrone (Fig. 2).

Discussion

Far from being typical NSAIDs, dipyrone and paracetamol are two similar non-opioid analgesics possessing very little anti-inflammatory action. Their central activity appears to mediate their analgesic and hypothermic effects in addition to their well-known peripheral effects. NOP receptor activation antagonised paracetamol-induced anti-nociception, whereas inhibition of these receptors was ineffective in preventing paracetamol-induced hypothermia (15,16). Here, unlike paracetamol, NOP receptor inhibition attenuated the anti-nociceptive action of dipyrone. On the other hand, similar to results obtained with paracetamol, we did not find any evidence that NOP receptor activation is involved in the hypothermic effect of dipyrone.

Pain-related central nervous system structures, such as the PAG, RVM and spinal cord, are known

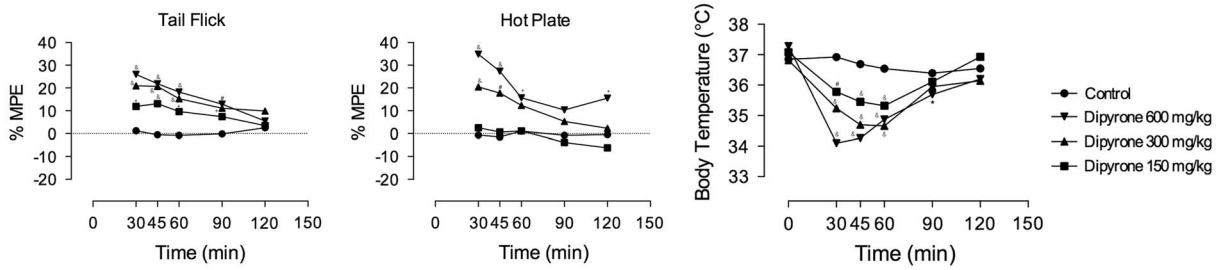

Fig. 1. Time course of the anti antinociceptive and hypothermic effects of dipyrone (150, 300, 600 mg/kg). (Compared to control: $^{*}p < 0.05$, $^{+}p < 0.01$, $p < 0.005$, $^{\&}p < 0.001$; n = 10 for each group; MPE = maximal possible effect).

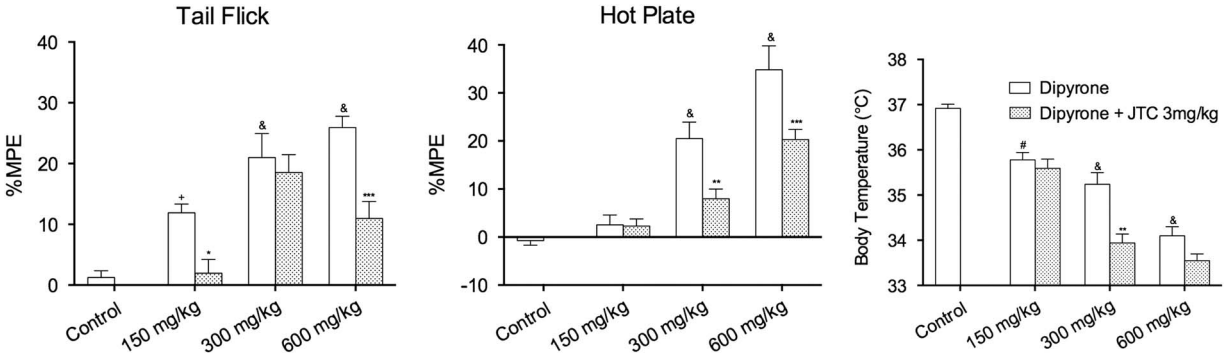

Fig. 2. Effects of the NOP receptor antagonist, JTC-801 (3 mg/kg), on the antinociceptive and hypothermic effects of dipyrone. JTC-801 was injected 30 min before, and tests were performed 30 min after dipyrone administration. (Compared to control: $^{+}p < 0.01$, $\#p < 0.05$, $^{\&}p < 0.001$; Compared to dipyrone: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$; n = 10 for each group; MPE = maximal possible effect).

https://doi.org/10.1017/neu.2014.38 Published online by Cambridge University Press

to mediate the anti-nociceptive effects of dipyrone not only when the drug is administered spinally or supra-spinally but also when administered systemically, pointing to the clinical relevance of this effect (3). Accordingly, facilitation of endogenous opioidergic circuits and activation of descending pain-control system, which inhibits pain transmission at the level of the spinal cord, are involved in this effect of dipyrone (3–5). Dipyrone is also proposed to be involved in interaction with the L-arginine-nitric oxide pathway, the glutamatergic system and the endogenous cannabinoid system, as well as its well-characterised effect of cyclooxygenase inhibition (8–23). Here, we observed whether NOP receptors participate in dipyrone-induced anti-nociception. Contrary to the results showing that NOP receptor activation prevents the anti-nociceptive action of paracetamol, our findings suggest that blockade of NOP receptors attenuates anti-nociception induced by dipyrone. Our results may be due to the complex role of the N/OFQ system in pain modulation, because whether N/OFQ has pro-nociceptive or anti-nociceptive properties and/or bidirectional-modulating actions still remain unknown (9,10). Supporting this complex modulation of pain by the N/OFQ system, both NOP receptor agonists and antagonists demonstrate potential utility in treating pain states (24,25). Therefore, obtaining contradictory results by administration of paracetamol and dipyrone, especially when the N/OFQ system is modulated, seems not to be unusual. Nevertheless, our findings indicating attenuation of dipyrone anti-nociception by the NOP antagonist are plausible, when it is taken into consideration that activation of NOP receptors has been associated with pain enhancement at some brain sites (26,27).

Regarding thermoregulation, we observed that NOP receptor antagonism did not reverse dipyrone-induced hypothermia. This effect is similar to that of paracetamol (16), whose hypothermic action has been studied more intensively than dipyrone. Paracetamol-induced hypothermia has been shown to be independent of the transient receptor vanilloid potential – 1 system and opioid, cannabinoid and NOP receptors (16–28). A cyclooxygenase-1 variant and its antioxidant and anti-glutamatergic properties are among the possible hypothermic mechanisms of paracetamol (29–31). In case of dipyrone, very little is known; it exerts hypothermia at high doses and its antipyretic mechanism is suggested not to involve PGE_2 synthesis inhibition (32,33). As described in the introduction, N/OFQ administration results in hypothermia, whereas NOP receptor knockout exerts hyperthermia (11,12). In contrast to these reports, we have observed that JTC-801 increased dipyrone-induced hypothermia at the 300 mg/kg dose of dipyrone. This unexpected finding may have resulted from differences in experimental procedures, species and strains of animals and other methodologies. Despite this discrepancy, our results indicate that, similar to its effect on paracetamol (16), NOP receptor antagonism seems not to reverse dipyrone-induced hypothermia.

In summary, we report that antagonising NOP receptors attenuates dipyrone-induced anti-nociception, but does not reverse dipyrone-induced hypothermia. The mechanisms through which dipyrone exerts its anti-nociceptive and hypothermic effects await further exploration, although it seems that they are mediated by dissimilar mechanisms.

Acknowledgement

Ismet Hande Ertin, MSc, contributed to the acquisition and interpretation of data, drafting the article and final approval of the version to be published. Associate Professor Dr. Ozgur Gunduz contributed to the acquisition, analysis and interpretation of data, drafting the article and final approval of the version to be published. Professor Dr. Ahmet Ulugol contributed to the conception, design, analysis and interpretation of data, drafting and revising the article for important intellectual content and final approval of the version to be published.

Financial Support

This work was supported by a grant from Trakya University Research Council (TUBAP-2013/32).

Conflicts of Interest

None.

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

References

1. Akman H, Aksu F, Gultekin I et al.. A possible central antinociceptive effect of dipyrone in mice. Pharmacology 1996;**53**:71–78.
2. Carlsson KH, Jurna I. The role of descending inhibition in the antinociceptive effects of the pyrazolone derivatives, metamizol (Dipyrone) and aminophenazone (Pyramidon). N-S Arch Pharmacol 1987;**335**:154–159.
3. Vazquez E, Hernandez N, Escobar W, Vanegas H. Antinociception induced by intravenous dipyrone (metamizol) upon dorsal horn neurons: involvement of

https://doi.org/10.1017/neu.2014.38 Published online by Cambridge University Press

endogenous opioids at the periaqueductal gray matter, the nucleus raphe magnus, and the spinal cord in rats. Brain Res 2005;**1048**:211–217.
4. Vanegas H, Tortorici V. Opioidergic effects of nonopioid analgesics on the central nervous system. Cell Mol Neurobiol 2002;**22**:655–661.
5. Hernandez-Delgadillo GP, Cruz SL. Endogenous opioids are involved in morphine and dipyrone analgesic potentiation in the tail flick test in rats. Eur J Pharmacol 2006;**546**:54–59.
6. Ulugol A. The endocannabinoid system as a potential therapeutic target for pain modulation. Balkan Med J 2014;**31**:115–120.
7. Escobar W, Ramirez K, Avila C, Limongi R, Vanegas H, Metamizol Vazquez E. A non-opioid analgesic, acts via endocannabinoids in the PAG-RVM axis during inflammation in rats. Eur J Pain 2012;**16**:676–689.
8. Rogosch T, Sinning C, Podlewski A et al. Novel bioactive metabolites of dipyrone (metamizol). Bioorgan Med Chem 2012;**20**:101–107.
9. Heinricher MM. Nociceptin/orphanin FQ: pain, stress and neural circuits. Life Sci 2005;**77**:3127–3132.
10. Mika J, Obara I, Przewlocka B. The role of nociceptin and dynorphin in chronic pain: implications of neuro-glial interaction. Neuropeptides 2011;**45**:247–261.
11. Rawls SM, Schroeder JA, Ding Z, Rodriguez T, Zaveri N. NOP receptor antagonist, JTC-801, blocks cannabinoid-evoked hypothermia in rats. Neuropeptides 2007;**41**:239–247.
12. Uezu K, Sei H, Sano A et al. Lack of nociceptin receptor alters body temperature during resting period in mice. Neuroreport 2004;**15**:751–755.
13. Mallet C, Daulhac L, Bonnefont J et al. Endocannabinoid and serotonergic systems are needed for acetaminophen-induced analgesia. Pain 2008;**139**:190–200.
14. Dogrul A, Seyrek M, Akgul EO, Cayci T, Kahraman S, Bolay H. Systemic paracetamol-induced analgesic and antihyperalgesic effects through activation of descending serotonergic pathways involving spinal 5-HT7 receptors. Eur J Pharmacol 2012;**677**:93–101.
15. Sandrini M, Vitale G, Pini LA, Lopetuso G, Romualdi P, Candeletti S. Nociceptin/orphanin FQ prevents the antinociceptive action of paracetamol on the rat hot plate test. Eur J Pharmacol 2005;**507**:43–48.
16. Corley G, Rawls SM. Opioid, cannabinoid CB1 and NOP receptors do not mediate APAP-induced hypothermia in rats. Pharmacol Biochem Behav 2009;**92**:503–507.
17. Gunduz O, Karadag HC, Ulugol A. Synergistic anti-allodynic effects of nociceptin/orphanin FQ and cannabinoid systems in neuropathic mice. Pharmacol Biochem Behav 2011;**99**:540–544.
18. Elmas P, Ulugol A. Involvement of cannabinoid CB1 receptors in the antinociceptive effect of dipyrone. J Neural Transm 2013;**120**:1533–1538.
19. Yilmaz I, Ulugol A. The effect of nitric oxide synthase inhibitors on the development of analgesic tolerance to dipyrone in mice. Int J Neurosci 2009;**119**:755–764.
20. Escobar W, Ramirez K, Avila C, Limongi R, Vanegas H, Metamizol Vazquez E. A non-opioid analgesic, acts via endocannabinoids in the PAG-RVM axis during inflammation in rats. Eur J Pain 2012;**16**:676–689.
21. Campos C, de Gregorio R, Garcia-Nieto R, Gago F, Ortiz P, Alemany S. Regulation of cyclooxygenase activity by metamizol. Eur J Pharmacol 1999;**378**:339–347.
22. Lorenzetti BB, Ferreira SH. Activation of the arginine-nitric oxide pathway in primary sensory neurons contributes to dipyrone-induced spinal and peripheral analgesia. Inflamm Res 1996;**45**:308–311.
23. Siebel JS, Beirith A, Calixto JB. Evidence for the involvement of metabotropic glutamatergic, neurokinin 1 receptor pathways and protein kinase C in the antinociceptive effect of dipyrone in mice. Brain Res 2004;**1003**:61–67.
24. Tamai H, Sawamura S, Takeda K, Orii R, Hanaoka K. Anti-allodynic and anti-hyperalgesic effects of nociceptin receptor antagonist, JTC-801, in rats after spinal nerve injury and inflammation. Eur J Pharmacol 2005;**510**: 223–228.
25. Khroyan TV, Polgar WE, Orduna J et al. Differential effects of nociceptin/orphanin FQ (NOP) receptor agonists in acute versus chronic pain: studies with bifunctional NOP/mu receptor agonists in the sciatic nerve ligation chronic pain model in mice. J Pharmacol Experimental Ther 2011;**339**:687–693.
26. Mogil JS, Grisel JE, Reinscheid RK, Civelli O, Belknap JK, Grandy DK. Orphanin FQ is a functional anti-opioid peptide. Neuroscience 1996;**75**:333–337.
27. Gear RW, Bogen O, Ferrari LF, Green PG, Levine JD. NOP receptor mediates anti-analgesia induced by agonist–antagonist opioids. Neuroscience 2014;**257**:139–148.
28. Ayoub SS, Pryce G, Seed MP, Bolton C, Flower RJ, Baker D. Paracetamol-induced hypothermia is independent of cannabinoids and transient receptor potential vanilloid-1 and is not mediated by AM404. Drug Metab Dispos 2011;**39**:1689–1695.
29. Ayoub SS, Botting RM, Goorha S, Colville-Nash PR, Willoughby DA, Ballou LR. Acetaminophen-induced hypothermia in mice is mediated by a prostaglandin endoperoxide synthase 1 gene-derived protein. Proc Nat Acad Sci U S A 2004;**101**:11165–11169.
30. Maharaj DS, Saravanan KS, Maharaj H, Mohanakumar KP, Daya S. Acetaminophen and aspirin inhibit superoxide anion generation and lipid peroxidation, and protect against 1-methyl-4-phenyl pyridinium-induced dopaminergic neurotoxicity in rats. Neurochem Int 2004;**44**:355–360.
31. Huang WT, Wang JJ, Lin MT. Antipyretic effect of acetaminophen by inhibition of glutamate release after staphylococcal enterotoxin A fever in rabbits. Neurosci Lett 2004;**355**:33–36.
32. Schlosburg JE, Radanova L, Di Marzo V, Imming P, Lichtman AH. Evaluation of the endogenous cannabinoid system in mediating the behavioral effects of dipyrone (metamizol) in mice. Behav Pharmacol 2012;**23**:722–726.
33. Malvar DD, Soares DM, Fabricio ASC et al. The antipyretic effect of dipyrone is unrelated to inhibition of PGE(2) synthesis in the hypothalamus. Brit J Pharmacol 2011;**162**:1401–1409.

https://doi.org/10.1017/neu.2014.38 Published online by Cambridge University Press