Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 503 (2004) 43–48

www.elsevier.com/locate/ejphar

Peripheral involvement of the nitric oxide–cGMP pathway in the indomethacin-induced antinociception in rat

Rosa Ventura-Martínez[a], Myrna Déciga-Campos[b], Ma. Irene Díaz-Reval[c], Ma. Eva González-Trujano[d], Francisco J. López-Muñoz[b,*]

[a]*Facultad de Medicina, Departamento de Farmacología, Universidad Nacional Autónoma de México, Ciudad Universitaria Coyoacán, C.P. 04510, México, D.F., México*

[b]*Lab. No. 7 "Dolor y Analgesia" del Departamento de Farmacobiología, CINVESTAV-IPN, Czda. de los Tenorios 235 Col. Granjas Coapa, Deleg. Tlalpan, C.P. 14330, México, D.F., México*

[c]*Centro Universitario de Investigaciones Biomédicas, Universidad de Colima. Av. 25 de julio No. 965, Col. Villa San Sebastián, C.P. 28045, Colima, Col., México*

[d]*Instituto Nacional de Psiquiatría "Ramón de la Fuente Muñiz", Av. México-Xochimilco No. 101, Col. Sn. Lorenzo Huipulco. C.P. 14370, México, D.F., México*

Received 30 August 2004; accepted 7 September 2004
Available online 5 October 2004

Abstract

The role of nitric oxide (NO) in the antinociceptive effect of indomethacin was assessed in the pain-induced functional impairment model in the rat (PIFIR model), a model of inflammatory and chronic pain similar to that observed in clinical gout. Oral administration of indomethacin (5.6 mg/kg), a nonselective cyclooxygenase inhibitor, significantly decreased the nociceptive response elicited by uric acid injected into the knee joint of the right hind limb (2.0±3.0 and 149.7±18.0 area units [au], in the absence and the presence of indomethacin, respectively). This effect of indomethacin was reduced in nearly 50% by local pretreatment with the nonselective inhibitor of NO synthase, N^G-L-nitro-arginine methyl ester (L-NAME) (72.9±10.7 vs. 149.7±18.0 au, $P<0.05$). On the other hand, local administration of L-arginine (a NO synthase substrate) or sodium nitroprusside (a non-enzymatic NO donor) each increased in almost 40% the antinociceptive effect of indomethacin (230.9±12.6 and 226.6±9.7 vs. 149.7±18.0 au, $P<0.05$), whereas D-arginine (the inactive isomer of arginine) had no effect on the indomethacin antinociceptive response (208.0±34.9 vs. 149.7±18.0 au). These results suggest that, the antinociceptive effect of indomethacin involves, at least in part, the NO–cyclic GMP pathway at peripheral level.
© 2004 Published by Elsevier B.V.

Keywords: PIFIR model; Indomethacin; Antinociception; Nitric oxide; (Rat)

1. Introduction

Nitric oxide (NO) is involved in a variety of physiological functions such as vasodilatation, macrophage cytotoxicity, central nervous system plasticity and nociceptive processing (Schuman and Madison, 1994; Moncada, 1997). NO is synthesized from L-arginine through the action of several NO synthase subtypes, and it activates soluble guanylate cyclase, which in turn gives rise to increased levels of cyclic GMP (cGMP) (Meller and Gebhart, 1993). Evidence has been provided to suggest that NO and cGMP play a role in antinociception since local administration of L-arginine was reported to produce antinociception in rats with carragenin-induced hyperalgesia, and this effect was blocked by NO synthesis inhibitors and soluble guanylate cyclase inhibitors (Duarte et al., 1990). In accordance with these findings, local administration of non-enzymatic NO donors was found to inhibit prostaglandin- and carragenin-induced hyperalgesia; this effect was prevented by guanylyl cyclase inhibitors but not by NO synthase inhibitors (Ferreira et al., 1991).

* Corresponding author. Tel.: +52 09 55 50612851; fax: +52 09 55 50812863.
E-mail address: flopezm@prodigy.net.mx (F.J. López-Muñoz).

0014-2999/$ - see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.ejphar.2004.09.018

It has been suggested, on the other hand, that inflammatory pain involves nociceptor sensitisation and that cyclic adenosine monophosphate (cAMP) may play an important role in this process (Ferreira and Nakamura, 1979). These observations support the hypothesis that a neuronal balance between cAMP and cGMP concentrations may be critical for the upward or downward functional regulation of nociceptors (Ferreira and Nakamura, 1979; Duarte et al., 1990). More recently, the antinociceptive effects of certain non-steroidal anti-inflammatory drugs (NSAIDs), such as dipyrone, diclofenac and ketorolac (Tonussi and Ferreira, 1994; Granados-Soto et al., 1995; López-Muñoz et al., 1996), and some preferential cyclooxygenase-2 inhibitors, such as rofecoxib (Déciga-Campos and López-Muñoz, 2003; 2004), were reported to involve activation of the L-arginine–NO–cyclic GMP pathway in addition to inhibition of prostaglandin synthesis.

On the basis of the above information, the present study was designed to investigate whether local activation of the NO–cGMP pathway is involved in the antinociceptive effect of indomethacin in the pain-induced functional impairment model in the rat (PIFIR model). The PIFIR model provides a model of inflammatory and chronic pain similar to that observed in clinical gout (López-Muñoz et al., 1993).

2. Materials and methods

2.1. Animals

Female Wistar rats [Crl:(WI)BR] weighing 180–200 g were used in this study. Female animals were employed in order to establish a comparison with our previous data, which were obtained using female rats (Déciga-Campos and López-Muñoz, 2003; 2004; López-Muñoz et al., 2004). The animals were housed in a temperature- and light-controlled room under a 12:12-h light/dark cycle (light on at 7:00 A.M.) with water and food provided ad libitum. Twelve hours before the experiments, food was withheld, but the animals had free access to tap water. Using this schedule, indomethacin was given through oral route. All experimental procedures followed the Guidelines on Ethical Standards for Investigations of Experimental Pain in Animals (Zimmermann, 1983), and were carried out according to a protocol previously approved by the local Animal Ethics Committee. The number of experimental animals was kept to a minimum and they were used only once.

2.2. Drugs

Uric acid, N^G-nitro-arginine methyl ester (L-NAME), L-arginine, D-arginine, sodium nitroprusside and indomethacin were purchased from Sigma (St. Louis, MO, USA); hidralazine was provided by Novartis Farmaceutica (Mexico city). Uric acid was suspended in mineral oil whereas indomethacin was dissolved in 1% Na_2CO_3; the other substances were dissolved in physiological saline.

2.3. Measurement of antinociceptive activity

The antinociceptive activity was measured using the PIFIR model as described previously (López-Muñoz et al., 1993). Pain was induced by injection of 50 μl of 30% uric acid into the knee joint of the right hind limb (i.art.), under light anaesthesia with ether. An electrode was attached to the plantar surface of each hind paw between the plantar pads. Rats were allowed to recover from anaesthesia and they were then placed on a stainless steel cylinder of 30 cm in diameter. The cylinder was rotated at 4 rpm forcing the rats to walk. The variable measured in this model was the time of contact between each of the rat's hind paws and the cylinder. When the electrode placed on the animal's paw made contact with the cylinder floor, a circuit was closed and the time that the circuit remained closed was recorded. The cylinder was rotated for 2-min periods, during which time recordings were made, allowing the rats to rest for 28 min between recording periods. Rats were forced to walk every 30 min during 6 h. After uric acid injection, the animals developed a progressive dysfunction of the injured limb. The time of contact of the injured hind limb reached a zero value at 2–2.5 h after the uric acid injection. Data are expressed as the percentage of the functionality index (FI%), i.e., the time of contact of the injected foot divided by the time of contact of the control left foot multiplied by 100. Once the functionality index was zero, the rats received several treatments as specified below. Antinociception was estimated as the recovery of the time of contact. For the purpose of this study, inducing nociception in the experimental animals was unavoidable. At the end of the experiments, animals were euthanised; this action avoided unnecessary suffering to the animals.

2.4. Experimental design

Once the functionality index reached zero different groups of rats received vehicle, 800 μg of L-NAME (an NO synthesis inhibitor), 400 μg of L-arginine (NO synthase substrate), 400 μg of D-arginine (inactive isomer of L-arginine), 400 μg of sodium nitroprusside (a non-enzymatic donor of NO), 800 μg of L-NAME+400 μg of L-arginine, 800 μg of L-NAME+400 μg of sodium nitroprusside or saline into the knee joint of the right hind limb (ipsilateral administration). On the other hand, other groups of rats received 800 μg of L-NAME, 400 μg of L-arginine or 400 μg of sodium nitroprusside intraarticulary into the knee joint of the left hind limb as controls (contralateral administration). Fifteen minutes later (see Díaz-Reval et al., 2002), all groups of rats received 5.6 mg/kg of indomethacin administered orally and they were evaluated in the PIFIR model for 4 h. Recovery of the functionality index was considered as expression of the antinociceptive

effect. Each treatment was given to six animals. Doses of each compound were selected in previous experiments in which dose–response curves (DRC) were determined (data not shown), and adequate controls were performed.

2.5. Data analysis

FI% vs. time curves were constructed for each treatment and the corresponding time course was obtained. Area under the curve (AUC) values were calculated for each treatment. The AUC was considered as an expression of the overall antinociceptive activity during the 4-h observation period (López-Muñoz et al., 1993) and it was obtained by the trapezoidal rule (Rowland and Toser, 1989). All values for each treatment are the mean±S.E.M. of six animals. AUC values for each treatment were compared by analysis of variance (ANOVA) followed by the Dunnett's *t*-test. Statistical significance was set at $P<0.05$.

3. Results

3.1. Antinociceptive effect of indomethacin

Oral administration of indomethacin produced a dose-dependent antinociceptive effect in the PIFIR model. At a dose of 5.6 mg/kg the drug induced an antinociceptive effect of 149.7±18.0 au. Since the maximum antinociceptive effect (AUC) that may be observed in the PIFIR model under the present experimental conditions is 375 au (López-Muñoz et al., 1993), the recovery of functionality produced by the above dose of indomethacin was 40% (data not shown). In order to investigate the role of the NO–cGMP pathway in the antinociceptive response to indomethacin, the dose of 5.6 mg/kg of the drug was selected.

3.2. Role of the NO–cGMP pathway in indomethacin-induced antinociception

Neither did ipsilateral (right hind limb) nor contralateral (left hind limb) injection with L-NAME (800 μg/art), L-arginine (400 μg/art), D-arginine (400 μg/articulation) or sodium nitroprusside (400 μg/art) after acid uric injection elicited any antinociceptive effect, i.e., dysfunction persisted during the entire observation period (data not shown). Interestingly, pretreatment with L-NAME (800 μg/art, ipsilateral) 15 min before indomethacin administration (5.6 mg/kg) significantly reduced the antinociceptive effect of indomethacin and this effect could be observed since the first hour of the observation period, as compared with rats pretreated with saline solution (S.S.). In marked contrast, pretreatment with L-arginine (400 μg/art, ipsilateral) increased the antinociceptive effect of indomethacin. However, the combined pretreatment with L-NAME (800 μg/art) and L-arginine (400 μg/art) reduced the antinociceptive effect to indomethacin (Fig. 1A).

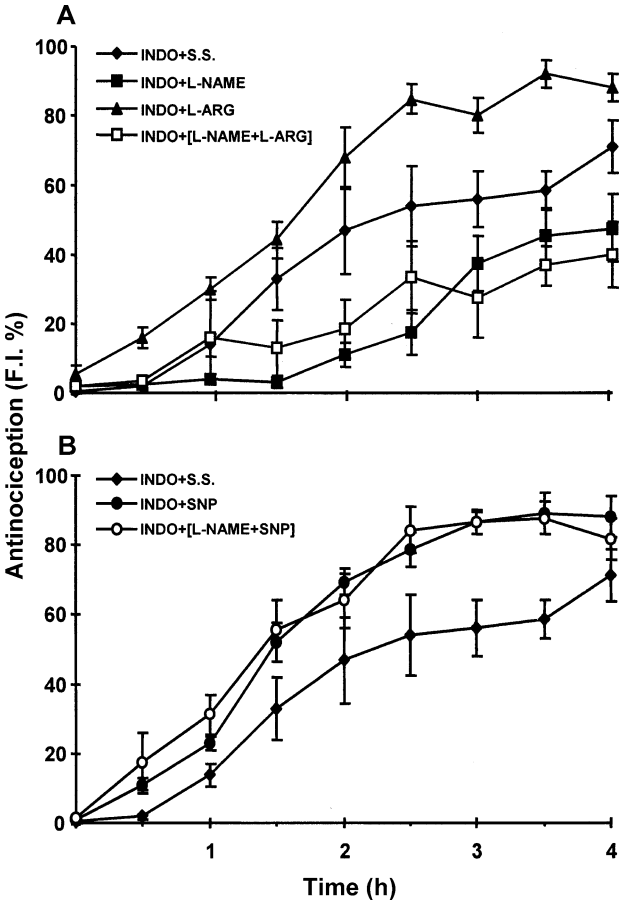

Fig. 1. (A) Time course of the antinociceptive effect induced by indomethacin (INDO; 5.6 mg/kg, p.o.) as measured as the recovery of the functionality index in rats receiving saline solution (S.S., ♦), 800 μg/art L-NAME (■), 400 μg/art L-arginine (L-ARG, ▲) or the combination of L-NAME+ L-arginine (□). (B) Time course of the antinociceptive effect induced by INDO (5.6 mg/kg, p.o.) as measured as the recovery of the functionality index in rats receiving saline solution (S.S., ♦), 400 μg/art sodium nitroprusside (SNP, ●) or the combination of L-NAME+SNP (○). Data are expressed as the mean±S.E.M. of six observations. The area under the curve (AUC) for each treatment was compared by analysis of variance (ANOVA) followed by the Dunnett's test.

On the other hand, sodium nitroprusside (400 μg/art, ipsilateral) increased significantly the antinociceptive effect of indomethacin, as compared with rats pretreated with saline solution (S.S.). Likewise, pretreatment with the combination of L-NAME (800 μg/art) and sodium nitroprusside (400 μg/art) increased the antinociceptive effect of indomethacin in an extent similar to that observed with sodium nitroprusside alone (Fig. 1B). When the global antinociceptive effect (AUC) of each treatment was analysed, it was observed that L-NAME (with or without L-arginine) significantly reduced the antinociceptive effect of indomethacin (68.0±16.1 or 72.9±10.7 vs. 149.7±18.0 au, Dunnett's test, $P<0.05$) and that L-arginine and sodium nitroprusside (with or without L-NAME) significantly increased the antinociceptive effect of indomethacin (230.9±12.6, 233.9±15.2 or 226.6±9.7 vs. 149.7±18.0 au, Dunnett's test, $P<0.05$) (Fig. 2).

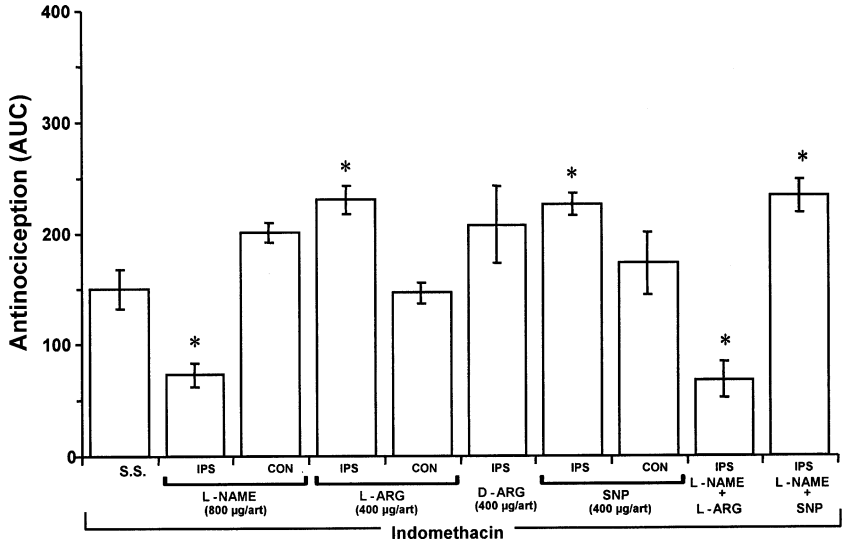

Fig. 2. Effect of several treatments on the global antinociceptive response produced by indomethacin (INDO; 5.6 mg/kg, p.o.). All compounds were given 15 min before INDO into the knee joint of the right (ipsilateral administration: IPS) or left (contralateral administration: CON) hind limb. Bars indicate the mean±S.E.M. of six rats. *Significantly different from saline group ($P<0.05$) as determined by analysis of variance followed by the Dunnett's test.

Finally, administration of L-NAME (800 µg/art), L-arginine (400 µg/art), or sodium nitroprusside (400 µg/art) in the contralateral paws did not have any effect on the antinociceptive response to indomethacin (201.0±8.6, 146.2±9.5 or 173.1±28.5 vs. 149.7±18.0 au). These results imply that the effects observed after ipsilateral administration were local. Similarly, local administration of D-arginine, the inactive isomer of L-arginine, in the ipsilateral paw failed to modify the antinociceptive effect of indomethacin (208.0±34.9 vs. 149.7±18.0 au), thus indicating that the effect of L-arginine in the antinociceptive response to indomethacin was stereospecific (Fig. 2).

4. Discussion

Indomethacin is a nonselective cyclooxygenase inhibitor, however, inhibition of prostaglandins synthesis does not completely explain indomethacin-induced antinociception. In this work, indomethacin-induced antinociception was blocked by the local administration of L-NAME, a non-selective inhibitor of the NO synthase. These results suggest that local NO release may play a role in the peripheral antinociceptive effect produced by indomethacin. A previous study from our laboratory reported that local administration of L-NAME failed to block the antinociceptive effect of indomethacin; however, in those experiments, L-NAME was given 1 h before indomethacin administration (López-Muñoz et al., 1996). Since such quite a long period of time may have allowed for L-NAME elimination, thus preventing its action on the indomethacin-induced effect, we decided to re-evaluate the effect of NO synthase inhibition on indomethacin-induced antinociception.

In support for a role of NO in the antinociceptive effect of indomethacin, L-arginine, but not its inactive isomer, D-arginine, increased indomethacin-induced antinociception in the PIFIR model. These results suggest that the limiting step in the local formation of NO is the presence of the natural substrate of nitric oxide synthase. These observations are in agreement with the results obtained with the combined treatment of L-NAME and L-arginine, since the effects with such a combination were essentially the same as those observed with L-NAME alone on indomethacin-induced antinociception. In addition, local administration of sodium nitroprusside, a non-enzymatic NO donor, did not produce any effect by itself but induced a significant increase of the antinociceptive effect of indomethacin. These results are in contrast with other studies showing that local administration of NO donors alone was able to induce antinociceptive responses (Duarte et al., 1990; Ferreira et al., 1991; Duarte et al., 1992; Cunha et al., 1999). This discrepancy in the effect of NO donors could be explained on the basis of differences in the intensity and type of the inflammatory stimuli employed, experimental conditions, and models of pain and animal species used. The role of NO derived from the NO synthase activity is additionally supported by the observation that L-NAME was incapable of altering the increase in indomethacin-induced antinociception by sodium nitroprusside since this later molecule does not require NO synthase activity to exert its effect.

The present data support the hypothesis that, in addition to prostaglandin synthesis inhibition, local NO synthesis is involved in the antinociceptive effect of indomethacin. The present results, however, do not allow us to exclude the involvement of other mechanisms in the antinociceptive activity of this drug since the drug was administered systemically. These results are in accordance with previous reports showing the participation of the NO–cGMP pathway in the antinociceptive effects produced by several NSAIDs, such as dipyrone (Lorenzetti and Ferreira, 1996), ketorolac

(Granados-Soto et al., 1995), diclofenac (Tonussi and Ferreira, 1994) and rofecoxib (Déciga-Campos and López-Muñoz, 2003; 2004). It is worth highlighting, however, that activation of the NO–cGMP pathway has been reported to result in hyperalgesia rather than antinociception. In these studies, local or systemic administration of L-NAME led to dose-dependent antinociceptive effects (Haley et al., 1992; Malberg and Yaksh, 1993; Aley et al., 1998). The nociceptive or inflammatory role of the NO–cGMP pathway has been described after its activation with bradykinin, substance P and carrageenin (Kabawata et al., 1994). Hence, the simplest explanation for these conflicting observations may be that the role of this pathway can vary among the groups of primary sensory neurones recruited by different types of nociceptive stimuli (Cunha et al., 1999).

In addition to the potential interaction with NO as a mechanism of antinociceptive activity, it is possible that sodium nitroprusside or L-arginine affect the AUC of indomethacin through a local effect on the vasculature and drug disposition. To exclude this possibility, we tested the effect of hidralazine, a peripheral vasodilator that does not require NO formation. This drug given intraarticularly (200 μg/articulation) did not produce any antinociceptive effect, and failed to alter the dysfunction induced by uric acid. Thus, these observations support the idea that the effect produced by sodium nitroprusside or L-arginine is mediated by a mechanism unrelated to vascular dilatation.

The triggering of the nociceptive process at peripheral level has been a subject of intense biomedical research for many years (Hill, 1999). The development of drugs exerting their antinociceptive effects by different mechanisms of action is an important strategy for reducing side effects in pain therapy. In this regard, potential therapeutic applications seem promising for nitric oxide-releasing non-steroidal anti-inflammatory drugs (NO-NSAID) as compared to typical NSAIDs (Keeble and Moore, 2002) since the former appear to cause markedly diminished gastrointestinal toxicity and have improved anti-inflammatory and antinociceptive efficacy. The present observations showing that a non-enzymatic donor of NO was able to increase the antinociceptive effect of indomethacin may suggest a therapeutic application for combinations of this kind.

In summary, this study demonstrated that indomethacin-induced antinociception was blocked by L-NAME and potentiated by L-arginine and sodium nitroprusside. These results strongly suggest that, besides the inhibitory action on prostaglandin synthesis inhibition, the activation of the NO–cGMP pathway may play an important role in the peripheral antinociceptive action of indomethacin.

Acknowledgements

We thank F. Sánchez, A. Huerta and L. Oliva for technical assistance.

References

Aley, K.O., McCerter, G., Levine, J.D., 1998. Nitric oxide signaling in pain and nociceptor sensitization in the rat. J. Neurosci. 18, 7008–7014.

Cunha, F.Q., Teixeira, M.M., Ferreira, S.H., 1999. Pharmacological modulation of secondary mediator systems—cyclic AMP and cyclic GMP—on inflammatory hyperalgesia. Br. J. Pharmacol. 127, 671–678.

Déciga-Campos, M., López-Muñoz, F.J., 2003. Participation of the NO–cyclic GMP pathway in rofecoxib-induced antinociception. Proc. West. Pharmacol. Soc. 46, 165–167.

Déciga-Campos, M., López-Muñoz, F.J., 2004. Participation of the L-arginine–nitric oxide–cyclic GMP–ATP-sensitive K+ channel cascade in the antinociceptive effect of rofecoxib. Eur. J. Pharmacol. 484, 193–199.

Díaz-Reval, M.I., Ventura-Martínez, R., Déciga-Campos, M., Terrón, J.A., Cabré, F., López-Muñoz, F.J., 2002. Involvement of serotonin mechanism in the antinociceptive effect of *S*(+)-ketoprofen. Drug Dev. Res. 57, 187–192.

Duarte, I.D.G., Lorenzetti, B.B., Ferreira, S.H., 1990. Peripheral analgesia and activation of the nitric oxide–cyclic GMP pathway. Eur. J. Pharmacol. 186, 289–293.

Duarte, I.D.G., Santos, I.R., Lorenzetti, B.B., Ferreira, S.H., 1992. Analgesia by direct antagonism of nociceptor sensitization involves the arginine–nitric oxide–cGMP pathway. Eur. J. Pharmacol. 217, 225–227.

Ferreira, S.H., Nakamura, M., 1979. Prostaglandin hyperalgesia: a cAMP/Ca+2-dependent process. Prostaglandins 18, 179–190.

Ferreira, S.H., Duarte, I.D.G., Lorenzetti, B.B., 1991. The molecular mechanism of action of peripheral morphine analgesia: stimulation of the cGMP system via nitric oxide release. Eur. J. Pharmacol. 201, 121–122.

Granados-Soto, V., Flores-Murrieta, F.J., Castañeda-Hernández, G., López-Muñoz, F.J., 1995. Evidence for the involvement of nitric oxide in the antinociceptive effect of ketorolac. Eur. J. Pharmacol. 277, 281–284.

Haley, J.E., Dickenson, A.H., Schachter, M., 1992. Electrophysiological evidence for a role of nitric oxide in prolonged chemical nociception in the rat. Neuropharmacology 31, 251–258.

Hill, R.G., 1999. Peripheral analgesic pharmacology: an update. In: Max, M. (Ed.), Pain 1999, An Update Review: Refresher Course Syllabus. International Association for the Study of Pain (IASP) Press, Seattle, pp. 391–395.

Kabawata, A., Manabe, S., Manabe, Y., Tagaky, H., 1994. Effect of topical administration of L-arginine on formalin-induced nociception in the mouse: a dual role of peripherally formed NO in pain modulation. Br. J. Pharmacol. 112, 547–550.

Keeble, J.E., Moore, P.K., 2002. Pharmacology and potential therapeutic applications of nitric oxide-releasing non-steroidal anti-inflammatory and related nitric oxide-donating drugs. Br. J. Pharmacol. 137 (3), 295–310.

López-Muñoz, F.J., Salazar, L.A., Castañeda-Hernández, G., Villarreal, J.E., 1993. A new model to assess analgesic activity: pain-induced functional impairment in the rat (PIFIR). Drug Dev. Res. 28, 169–175.

López-Muñoz, F.J., Castañeda-Hernández, G., Torres-López, J.E., Picazo, Y.F., Flores-Murrieta, F.J., Granados-Soto, V., 1996. Differences in the mechanism of antinociceptive action of non-steroidal anti-inflammatory drugs in the rat. Pharm. Sci. 2, 189–190.

López-Muñoz, F.J., Díaz-Reval, M.I., Terrón, J.A., Déciga-Campos, M., 2004. Analysis of the analgesic interactions between ketorolac and tramadol during arthritic nociception in rat. Eur. J. Pharmacol. 484, 157–165.

Lorenzetti, B.B., Ferreira, S.H., 1996. Activation of the arginine–nitric pathway in primary sensory neurons contributes to dipyrone-induced spinal and peripheral analgesia. Inflamm. Res. 45, 308–311.

Malberg, A.B., Yaksh, T.L., 1993. Spinal nitric oxide synthesis inhibition blocks NMDA-induced thermal hyperalgesia and produces antinociception in the formalin test. Pain 54, 291–300.

Meller, S.T., Gebhart, G.F., 1993. Nitric oxide (NO) and nociceptive processing in the spinal cord. Pain 52, 127–136.

Moncada, S., 1997. Nitric oxide in the vasculature: physiology and pathophysiology. Ann. N. Y. Acad. Sci. 811, 60–67.

Rowland, M., Toser, T.N., 1989. Clinical Pharmacokinetics: Concepts and Applications, 2nd ed. Lea and Febiger, Philadelphia, pp. 115–118.

Schuman, E.M., Madison, D.V., 1994. Nitric oxide and synaptic function. Annu. Rev. Neurosci. 17, 153–183.

Tonussi, C.R., Ferreira, S.H., 1994. Mechanism of diclofenac analgesia: direct blocked of inflammatory sensitization. Eur. J. Pharmacol. 251, 173–179.

Zimmermann, M., 1983. The guidelines on ethical standards for investigation of experimental pain in animals. Pain 16, 109–110.