Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 483 (2004) 241–248
www.elsevier.com/locate/ejphar

Evidence for a central mechanism of action of *S*-(+)-ketoprofen

María Irene Díaz-Reval[a,*], Rosa Ventura-Martínez[b], Myrna Déciga-Campos[b], José A. Terrón[c], Francesc Cabré[d,1], Francisco Javier López-Muñoz[b,*]

[a] *Centro Universitario de Investigaciones Biomédicas, Universidad de Colima, Av. 25 de Julio No. 965, Col. Villa San Sebastián, C.P. 28045 Colima, Col., Mexico*
[b] *Lab. No. 7 "Dolor y Analgesia" del Departamento de Farmacobiología, CINVESTAV-IPN, Calz. de los Tenorios No. 235, Col. Granjas Coapa, Deleg Tlálpan, C.P. 14330 México, D.F., Mexico*
[c] *Sección Externa de Farmacología, CINVESTAV-IPN, México, D.F., Mexico*
[d] *Menarini Research, Barcelona, Spain*

Received 16 June 2003; received in revised form 22 October 2003; accepted 28 October 2003

Abstract

It has been observed that some non-steroidal anti-inflammatory drugs (NSAIDs) may act through several mechanisms, in addition to central inhibition of prostaglandin synthesis. These other mechanisms include the L-arginine-nitric oxide (L-arginine-NO) pathway, as well as endogenous opiate and serotonergic mechanisms. Some of these mechanisms can explain the efficacy of NSAIDs in chronic pain conditions such as rheumatoid arthritis. The present study was designed to elucidate the involvement of the above pathways/mechanisms in the antinociceptive effect of *S*-(+)-ketoprofen at supraspinal and spinal levels. *S*-(+)-ketoprofen induced dose-dependent antinociception in the pain-induced functional impairment model in the rat. The antinociceptive effect of *S*-(+)-ketoprofen was not altered by i.t. or intracerebroventricula (i.c.v.) pre-treatment with L-arginine (29.6 μg/site) and L-nitro-arginine-monomethylester (L-NAME) (21.1 μg/site) and neither was the effect of *S*-(+)-ketoprofen modified by the opiate antagonist, naloxone (1 mg/kg, s.c.). In marked contrast, both i.c.v. administration of the 5-hydroxytryptamine (5-HT)$_1$/5-HT$_2$/5-HT$_7$ receptor antagonist, methiothepin (1.5 μg/site), and i.t. administration of the 5-HT$_3$/5-HT$_4$ receptor antagonist, tropisetron (0.9 μg/site), significantly inhibited the *S*-(+)-ketoprofen-induced antinociceptive effect. These data suggest that the antinociceptive response to *S*-(+)-ketoprofen involves serotoninergic mechanisms via both supraspinal 5-HT$_1$/5-HT$_2$/5-HT$_7$ receptors and 5-HT$_3$ receptors located at spinal level. A role of the L-arginine-NO and opiate systems in *S*-(+)-ketoprofen-induced antinociception in the pain-induced functional impairment model in the rat model seems unlikely.

Keywords: *S*-(+)-ketoprofen; Antinociception; Tropisetron; Methiothepin; 5-HT receptor antagonist

1. Introduction

Ketoprofen (2-[3-benzoylphenyl] propionic acid) is a non-steroidal anti-inflammatory agent (NSAID) with analgesic, anti-inflammatory and antipyretic properties, and is effective for the treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis (O'Brien and Grunwaldt, 1976; Famaey and Colinet, 1976; Caroit et al., 1976). The drug is an aryl carboxylic acid derivative with a chiral cent, which yields two enantiomeric forms, i.e. *R* and *S*. It has been reported that *S*-(+)-ketoprofen produces effective analgesic effects (Cabré et al., 1998). Its *S* isomer inhibits the synthesis of prostaglandin in vitro (Suesa et al., 1993; Carabaza et al., 1996) and the drug is more efficacious than diclofenac and indomethacin with regard to analgesic, antipyretic and anti-inflammatory activity (Cabré et al., 1998). Interestingly, consistent with the previous observations suggesting both peripheral and central sites of action for *S*-(+)-ketoprofen (Beltran et al., 1998), i.c.v. administration of this compound produced similar effects to those induced by morphine in the pain-induced functional impairment model in the rat (López-Muñoz et al., 1988). These observations support the notion that *S*-(+)-ketoprofen-induced antinociception involves other mechanisms in addition to inhibition of prostaglandin synthesis. Actually, it has

* Corresponding authors. Tel.: +52-9-55-5061-2851, +52-9-312-3161129; fax: +52-9-55-5061-2863.
E-mail addresses: flopezm@prodigy.net.mx (F.J. López-Muñoz), idiazre@cgic.ucol.mx (M.I. Díaz-Reval).
[1] Present address: Preclinical Pharmacology, Vita-Invest S.A.

0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2003.10.036

been reported that the analgesic effects of diclofenac involve the participation of other mechanisms, i.e. L-arginine-nitric oxide pathway and serotonergic system (Björkman, 1995).

It has been shown that depolarisation of afferent neurones by peripheral harmful stimuli leads to activation of spinal NMDA receptors. These, in turn, promote the synthesis of nitric oxide (NO), which is a neurotransmitter at a spinal level conveying nociceptive information (Inoue et al., 1998; Meller et al., 1992). The role of NO in nociception is evidenced by the fact that some NSAIDs, such as *S*-(+)-ibuprofen, inhibit NO synthesis (Björkman, 1995). In addition, experimental observations suggest that endogenous opiates may be involved in the analgesic effects of some NSAIDs. For instance, Björkman et al. (1990) reported that the antinociceptive effect of diclofenac was reversed by s.c. administration of naloxone in rats, whereas an increase in the plasma levels of β-endorphins was noticed in humans (Sacerdote et al., 1985). An additional mechanism probably involved in NSAID-induced analgesia is the serotonin (5-hydroxytryptamine; 5-HT) system, which modulates the pain pathway (Yaksh and Wilson, 1979; Bardin et al., 2000). Indeed, acetylsalicylic acid was reported to increase the brain 5-HT content in rats, and its antinociceptive effects were abolished by naloxone pre-treatment (Pini et al., 1997a,b).

On the basis of the above observations, the present study was aimed at elucidating the potential involvement of the L-arginine-nitric oxide (L-arginine-NO) pathway, and the opiate and serotonergic systems, both peripherally and centrally, in the analgesic effect of *S*-(+)-ketoprofen in the pain-induced functional impairment model in the rat.

2. Materials and methods

2.1. Animals

Male Wistar rats, 200–220 g, from our own breeding facilities [Crl(WI)BR] were used in this study. The animals were housed in a room with controlled temperature (22 ± 2 °C) with a 12-h alternating light/dark cycle. Twelve hours before experiments, food was withheld, but the animals had free access to tap water. All experimental procedures followed the recommendations of "The Committee for Research and Ethical Issues of the International Association for the Study of Pain" (Covino et al., 1980), and the guidelines on ethical standards for investigations of experimental pain in animals (Zimmermann, 1983), and were carried out according to a protocol approved by the local Animal Ethics Committee. The number of experimental animals was kept to a minimum.

2.2. Measurement of antinociceptive activity

Antinociceptive activity was measured using the pain-induced functional impairment model in the rat, which has been previously described in detail (López-Muñoz et al., 1993). Briefly, the animals were anaesthetised with ether, and 0.05 ml of 30% uric acid suspended in mineral oil was administered by intra-articular (i.art.) injection into the knee joint of the right hind limb to induce nociception. An electrode was attached to each hind paw between the plantar pads. When the animals recovered from anaesthesia, they were placed on a stainless steel cylinder of 30-cm diameter. The cylinder was rotated at 4 rpm, so the rats were forced to walk. When the animal's paw electrode made contact with the surface of the cylinder, a circuit was closed and the time of contact between each rat's hind paw and the cylinder was recorded with a specially designed computer program. The cylinder rotated for periods of 2 min, during which time recordings were made. Rats were allowed to rest for 30 min between recording periods.

After uric acid injection, the animals developed a progressive dysfunction of the injured limb. The time of contact of the injured hind paw reached a zero value at 2–2.5 h after uric acid injection. At this time, the rats received an oral dose of *S*-(+)-ketoprofen (0.20, 0.34, 1.08, 3.4, 10.8, 18.9, 34.0 and 71.0 mg/kg). The periods of contact were stet every 30 min during the following 4 h. Antinociception was estimated as the recovery of the contact time. Data are expressed as the functionality index (FI) in percentage according to the following equation:

$$\%\mathrm{FI} = [(\text{time of contact of injured right paw}) / (\text{time of contact of control left paw})](100)$$

2.3. Intrathecal injections

The procedure used was similar to that previously described by Yaksh and Rudy (1976). Briefly, rats were chronically implanted with catheters; they were anaesthetised with pentobarbital (40 mg/kg), placed in a stereotaxic frame, and submitted to surgical procedures to expose the atlanto occipital membrane. This was pierced and the tip of a PE-10 catheter was inserted through the *cisterna magna* and extended 8.0 cm. The incisions were sutured and 8 days was allowed for recovery. The day of experiment, the animals received a volume of 10 μl.

2.4. Intracerebroventricular injections

Intracerebroventricular (i.c.v.) injections were performed according to the method described by Paxinos and Watson (1982). Briefly, rats were anaesthetised with pentobarbital (40 mg/kg) 24 h before the experiments and placed in a stereotaxic frame. Immediately afterwards, a cannula was inserted into the right lateral ventricle (coordinates: dorsoventral 3.9 mm, lateral 1.4 mm and anteroposterior 0.8 mm). The day of the experiment animals

were administered, the corresponding antagonist drugs contained in a volume of 4 μl.

2.5. Study design

To determine the possible involvement of central mechanisms in the antinociceptive effect of *S*-(+)-ketoprofen, inhibitors and antagonist drugs were administered via i.c.v., i.t. and s.c. routes. Thus, to elucidate the role of the opiate system, animals were pre-treated with the opioid receptor antagonist, naloxone (1 mg/kg, s.c.). To assess the possible participation of the L-arginine-NO pathway in the analgesic effect of *S*-(+)-ketoprofen, different groups of animals were pre-treated intrathecally and intracerebroventricularly with L-arginine (21.1 μg/site). Other groups of rats were pre-treated with the NO synthase inhibitor, L-nitro-arginine-monomethylester (L-NAME; 26.9 μg/site, i.t. and i.c.v.). Finally, to determine the role of the 5-HT system in *S*-(+)-ketoprofen-induced antinociception, other groups of animals were given the 5-HT_1/5-HT_2/5-HT_7 receptor antagonist, methiothepin (1.5 μg/site), by both i.t. and i.c.v. routes, and the 5-HT_3/5-HT_4 receptor antagonist, tropisetron (0.9 μg/site, i.t. and i.c.v.). After a period of 15 min, all groups were administered *S*-(+)-ketoprofen (3.4 mg/kg, p.o.) and the antinociceptive activity was measured. In control experiments, other groups of animals received *S*-(+)-ketoprofen (3.4 mg/kg, p.o.), morphine (56.2 mg/kg p.o.), naloxone (1 mg/kg, s.c.), L-arginine (21.1 μg/site, i.t. and i.c.v.), L-NAME (26.9 μg/site, i.t. and i.c.v.), methiothepine (1.5 μg/site, i.t. and i.c.v.) and tropisetron (0.9 μg/site, i.t. and i.c.v.) alone.

2.6. Drugs

The thromethamine salt of *S*-(+)-ketoprofen was generously supplied by Farma Lepori (Barcelona Spain). Uric acid, L-arginine, L-NAME, and naloxone hydrochloride were purchased from Sigma (St. Louis, MO,. USA). Methiothepin mesylate salt and tropisetron hydrochloride were purchased from Research Biochemicals International, USA. *S*-(+)-ketoprofen was suspended in carboxymethylcellulose, whereas the other substances were dissolved in physiological saline.

2.7. Data presentation and statistical analyses

The antinociceptive effect is expressed as recovery of FI, which was plotted vs. time. The area under the curve (AUC) of the FI (i.e. the cumulative antinociceptive effect during the 4-h observation period, estimated by the trapezoidal rule) was plotted vs. dose or treatment. All data in the text and figures are expressed as the means ± S.E.M. of four and six experimental observations for i.t. and i.c.v. administration, respectively. The antinociceptive effect in each group was measured and compared by using Student's *t*-test. Statistical significance was defined at $P < 0.05$.

3. Results

3.1. Antinociceptive activity of S-(+)-ketoprofen

Fig. 1 (Panel A) shows the dose–response curve of *S*-(+)-ketoprofen after p.o. administration. The *Y*-axis depicts the total antinociceptive effect of the drug over the 4-h observation period, which is expressed as the AUC of the time course. The *X*-axis shows the dose of the drug in mg/kg. Eight doses of *S*-(+)-ketoprofen were tested (0.20 to 71.0 mg/kg). As can be seen, the effect of *S*-(+)-ketoprofen was dose dependent; the maximal effect was 273.6 ± 15.9 area units (au). The maximal AUC value that can be attained in this model was 375 au (dashed line).

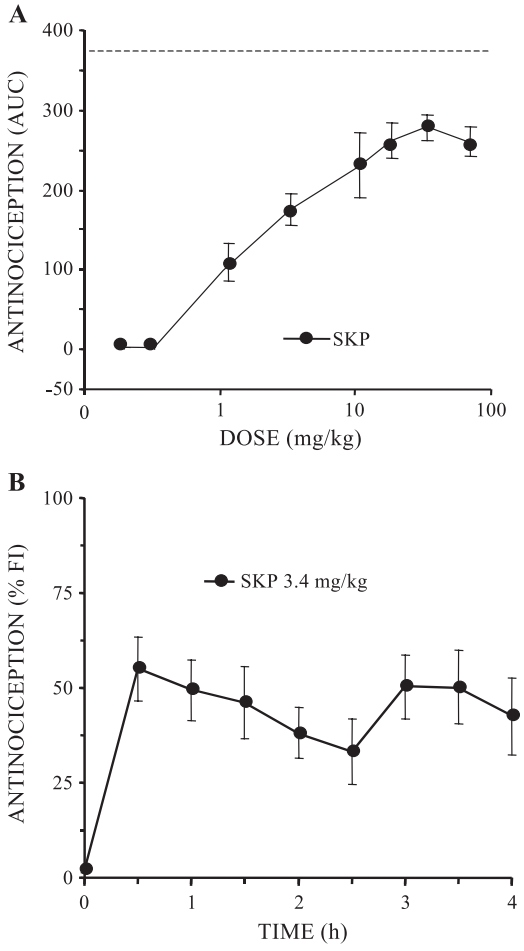

Fig. 1. (Panel A) Dose–response curve for the antinociceptive effect of *S*-(+)-ketoprofen. The *Y*-axis depicts the total antinociceptive effect of the drug during the 4-h observation period, expressed as the AUC of the time course. The *X*-axis depicts the dose of the drug in mg/kg. *S*-(+)-ketoprofen was administered p.o. in eight doses (0.20 to 71.0 mg/kg). The antinociceptive effect was dose dependent. The dashed line represents the maximal theoretical AUC value that can be attained under these experimental conditions. (Panel B) Time course of *S*-(+)-ketoprofen administered at the dose of 3.4 mg/kg. The *Y*-axis depicts the antinociceptive effect, expressed as a percentage of the functionality index. The *X*-axis represents the time in hours. The mean of six determinations ± S.E.M. is presented.

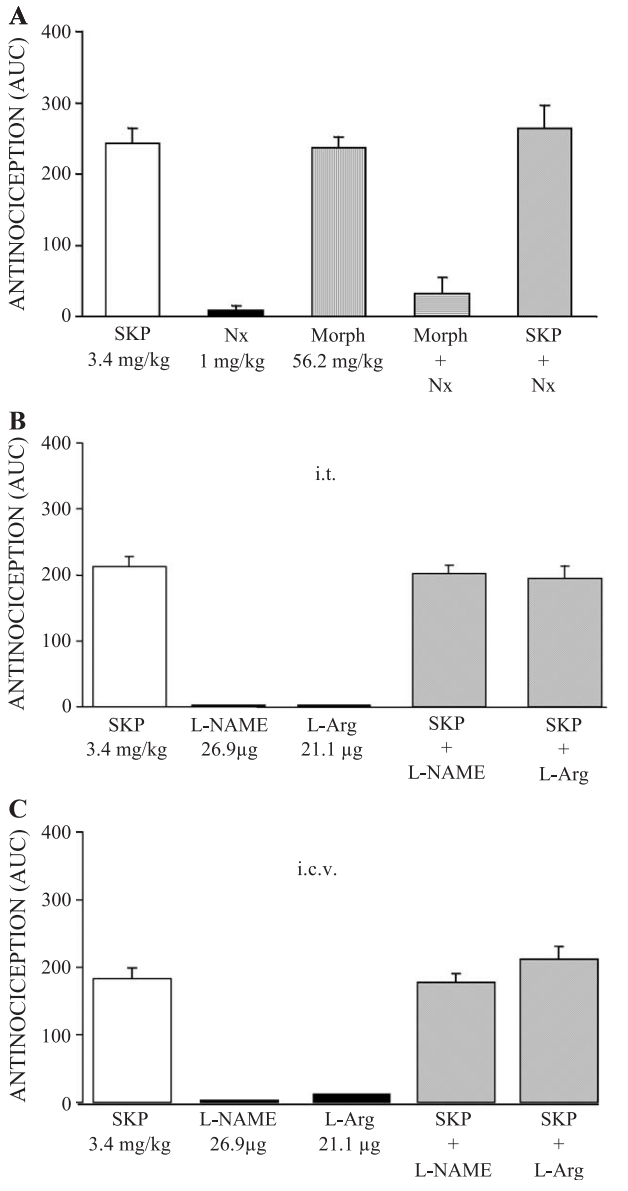

Fig. 2. (Panel A) The antinociceptive effect (expressed as AUC) in animals that received *S*-(+)-ketoprofen p.o. (SKP), naloxone s.c. (Nx), naloxone 15 min before *S*-(+)-ketoprofen, morphine p.o. (Morph) or naloxone 15 min before morphine. The effects of L-NAME and L-arginine (L-Arg) 15 min before administration of carboxymethylcelullose (the vehicle of *S*-(+)-ketoprofen) or *S*-(+)-ketoprofen (SKP) are also shown. (Panel B) I.t. administration of L-NAME and L-Arg; each column represents the mean ± S.E.M. for four animals. (Panel C) I.c.v. administration of L-NAME and L-Arg; each column represents the mean ± S.E.M. for six animals.

The dose of 3.4 mg/kg of *S*-(+)-ketoprofen was selected because it produced a response that allowed the observation of either inhibitory or excitatory effects of the drug treatments under study. Fig. 1 (Panel B) shows the time course of *S*-(+)-ketoprofen administered at the dose of 3.4 mg/kg: the maximum effect was 55.1 ± 8.4% FI, which was obtained at 30 min, the AUC was 172.5 ± 18.4 au. It should be noted that the effect was sustained throughout the entire experimental period.

3.2. Analysis of potential mechanisms of action of S-(+)-ketoprofen

Fig. 2 (Panel A) shows antinociceptive responses in animals treated with naloxone (1 mg/kg, s.c.); results are expressed as antinociception (AUC, *Y*-axis) vs. treatment (*X*-axis). As can be noticed, administration of naloxone 15 min before *S*-(+)-ketoprofen had no effect on the antinociceptive response. The same dose of naloxone (1 mg/kg, s.c.), in contrast, almost completely inhibited the analgesic effect of morphine 56.2 mg/kg p.o.

The i.t. administration of L-arginine (21.1 µg/site) and L-NAME (26.9 µg/site) 15 min before the administration of carboxymethylcelullose (the vehicle of *S*-(+)-ketoprofen) had no antinociceptive effect (Fig. 2, Panel B). *S*-(+)-ketoprofen (3.4 mg/kg, p.o.), in contrast, produced a clear and consistent antinociceptive effect (212.3 ± 15.6 au).

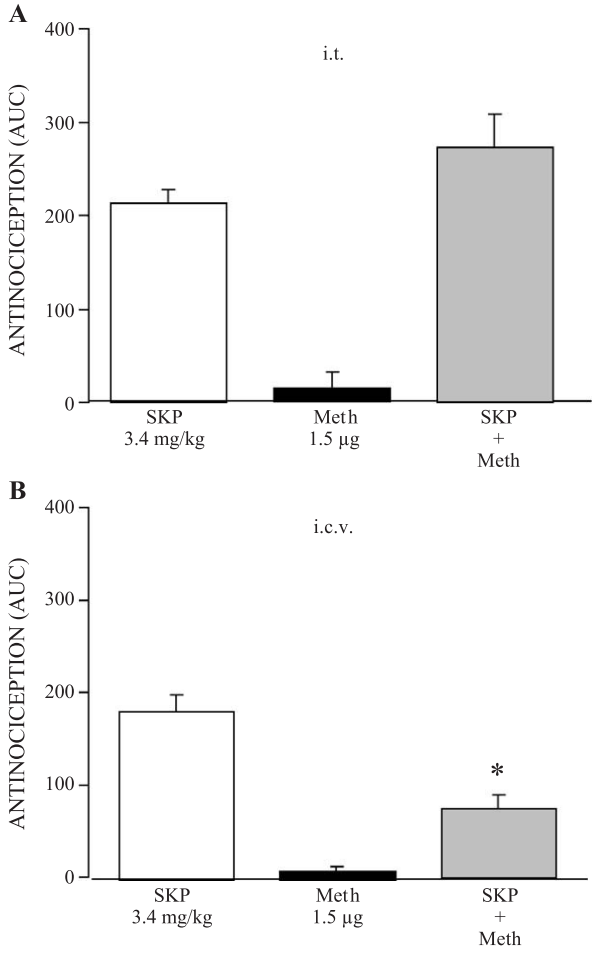

Fig. 3. The effect of methiothepin (1.5 µg/site: Meth) 15 min before administration of *S*-(+)-ketoprofen (3.4 mg/kg, p.o.: SKP). The open columns represent the antinociceptive effect of *S*-(+)-ketoprofen; dark columns represent the effect of methiothepin; and the hatched columns represent the effect of methiothepin + *S*-(+)-ketoprofen. (Panel A) I.t. administration of antagonist; values are presented as the means ± S.E.M. for four animals. (Panel B) I.c.v. administration of methiothepin; values are presented as the means ± S.E.M. for six animals. *$P < 0.05$, significantly different from *S*-(+)-ketoprofen.

This response was unaffected by the i.t. administration of either L-arginine (21.1 μg/site, 195.0 ± 19.1 au) or L-NAME (26.9 μg/site, 201.5 ± 12.9 au). The i.c.v. administration of L-arginine (21.1 μg/site) and L-NAME (26.9 μg/site) did not induce antinociceptive effects (Fig. 2, Panel C). Similar to the observations after i.t. treatment, i.c.v. administration of L-arginine (211.4 ± 44.7 au) or L-NAME (177.4 ± 23.0 au) failed to significantly alter the antinociceptive effect produced by *S*-(+)-ketoprofen (3.4 mg/kg, p.o. 182.8 ± 18.6 au).

To determine the role of the 5-HT system in *S*-(+)-ketoprofen-induced antinociception, the effects of methiothepin were analysed at a spinal level (Fig. 3, Panel A). I.t. administration of methiothepin (1.5 μg/site) 15 min before *S*-(+)-ketoprofen had an effect of 273.3 ± 35.1 au, which was not significantly different from that of the group that received *S*-(+)-ketoprofen alone (3.4 mg/kg, p.o., 212.3 ± 15.6 au). Administration of methiothepin alone did not produce antinociceptive effects. In contrast, i.c.v. injection of methiothepin (1.5 μg/site; Fig. 3, Panel B) 15 min before *S*-(+)-ketoprofen significantly inhibited *S*-(+)-ketoprofen-induced antinociception (AUC of 75.1 ± 14.7 au), as compared with that of the control group(182.8 ± 18.6 au).

As shown in Fig. 4 (Panel A), i.t. administration of tropisetron (0.9 μg/site) 15 min before carboxymethylcellulose did not produce antinociceptive effects; in contrast, the drug significantly inhibited the antinociceptive effect induced by *S*-(+)-ketoprofen (3.4 mg/kg, p.o.; 212.3 ± 15.6 and 85.9 ± 6.9 au in the absence and the presence of tropisetron, respectively). Interestingly, when tropisetron (0.9 μg/site) was given by the i.c.v. route, it failed to significantly modify *S*-(+)-ketoprofen-induced antinociception (182.8 ± 18.6 and 219.9 ± 27.8 au in the absence and the presence of tropisetron, respectively; Fig. 4, Panel B).

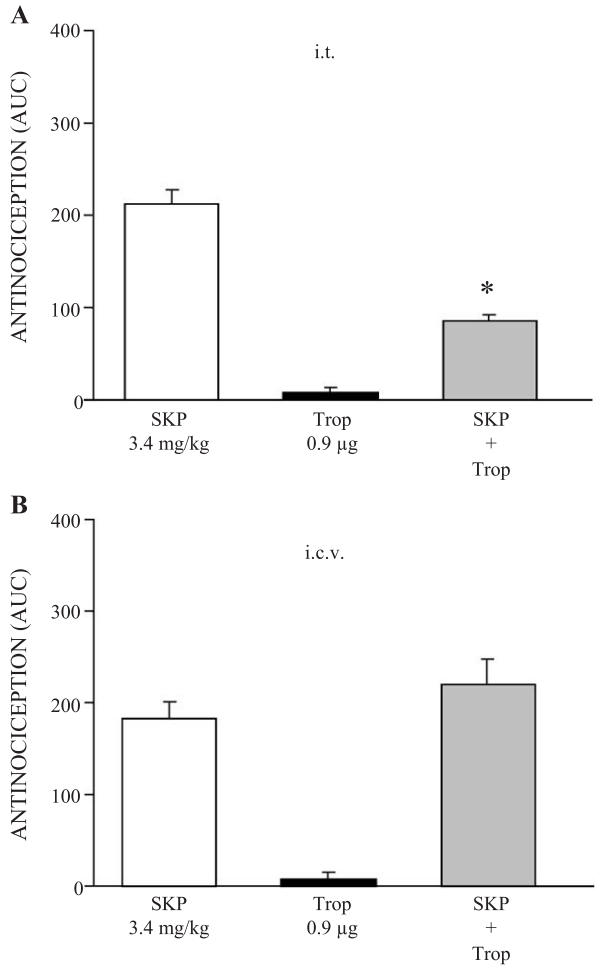

Fig. 4. The effects of tropisetron (0.9 μg/site: Trop) given 15 min before administration of *S*-(+)-ketoprofen (3.4 mg/kg, p.o.: SKP). The open columns represent the antinociceptive effect of *S*-(+)-ketoprofen; dark columns represent the effect of tropisetron; and hatched columns represent the effect of tropisetron + *S*-(+)-ketoprofen. (Panel A) I.t. administration of antagonist; values are presented as the means ± S.E.M. for four animals. (Panel B) I.c.v. administration of tropisetron; values are presented as the means ± S.E.M. for six animals. *$P < 0.05$, significantly different from *S*-(+)-ketoprofen.

4. Discussion

The "pain-induced functional impairment model in the rat" is an experimental model of inflammatory and chronic pain similar to that of clinical gout. Pain is produced by intra-articular administration of uric acid in the knee joint of the right hind limb of the rat. This model is sensitive to the antinociceptive action of both opiate and non-opiate analgesics. *S*-(+)-ketoprofen is a NSAID and exhibited dose-dependent effects, the maximal antinociceptive response being reached with a dose of 34.0 mg/kg; this corresponds to 73% of the maximum AUC value (375 area units) that can be attained under the present experimental conditions. This efficacy of *S*-(+)-ketoprofen is similar to that of morphine (177.8 mg/kg, p.o. produced 310.8 ± 12.0 au). The present observations are in agreement with the data reported previously in other experimental models, including clinical tests, showing a suitable efficacy of *S*-(+)-ketoprofen (Cabré et al., 1998; Ezcurdia et al., 1998; Bagán et al., 1998).

S-(+)-ketoprofen was administered by p.o. route because this is the administration route in humans. Indeed, it has been demonstrated that the racemic mixture of ketoprofen is highly lipophilic, so it can be detected in serum and cerebrospinal fluid 15 min after intramuscular administration (Netter et al., 1985). This implies that *S*-(+)-ketoprofen readily penetrates the blood–brain barrier (Netter et al., 1985; Barbanoj et al., 1998) and that it may activate mechanisms other than prostaglandin synthesis at a central level.

Central opiate mechanisms were analysed in the first place by testing the effects of s.c. naloxone on *S*-(+)-ketoprofen-induced antinociception. The dose of naloxone used in the present experiments (1 mg/kg, s.c.) was high enough to block opiate receptors, as demonstrated previ-

ously in the pain induced-functional impairment model, where the same dose of naloxone significantly decreased the antinociceptive effect of morphine, most likely due to blockade of μ, δ and κ receptors (Yaksh, 1997). In the present experiments, however, naloxone was completely inactive as antagonist, thus precluding the involvement of central opiate mechanisms in the antinociceptive effect of *S*-(+)-ketoprofen. It has been reported that activation of nociceptive C-fib afferents will cause the release of neurotransmitters, such as glutamate, within the dorsal horn. This in turn will activate NMDA receptors to promote NO synthesis, and NO is well known to function as a transmitter of pain in the spinal cord (Garthwaite et al., 1988; Garthwaite, 1991; Dickenson et al., 1997). Several lines of evidence suggest that some NSAIDs interact with this mechanism to produce their antinociceptive effects (Hunskaar et al., 1985; Björkman, 1995). However, as demonstrated in the present study, pre-treatment with the substrate of nitric oxide synthase, L-arginine, or with the inhibitor of nitric oxide synthase, L-NAME, either by i.t. or i.c.v. routes, failed to significantly modify the antinociceptive effect of *S*-(+)-ketoprofen, thereby excluding the participation of the L-arginine-NO pathway in *S*-(+)-ketoprofen-induced antinociception. The doses of L-arginine and L-NAME used in our experiments have been observed to alter the antinociceptive effect induced by other NSAIDs under the same experimental conditions (data no shown), and to be efficacious in modifying L-arginine-NO pathway-mediated effects in other experimental models (Meller et al., 1992).

5-HT is considered a neurotransmitter of the utmost significance in endogenous pain control mechanisms. Several lines of evidence suggest that the antinociceptive effect of various analgesics, either narcotic or non-narcotic, depends on the integrity of the central 5-HT system. For example, i.t. injection of a 5-HT receptor antagonist attenuated the analgesia produced by microinjection of morphine into the periaqueductal gray (Yaksh, 1979). Likewise, administration of *p*-chloro-phenylalanine, a tryptophan hydroxylase inhibitor, strongly interfered with the antinociceptive effect of NSAIDs, such as diclofenac and acetaminophen (Björkman, 1995; Pini et al., 1996). In spinal analgesia, a body of experimental evidence (Bardin et al., 2000; Cesselin et al., 1994; Le Bars, 1988; Yaksh and Wilson, 1979) also supports a role of 5-HT.

In our experiments, i.c.v. administration of the high-affinity 5-HT_1/5-HT_2/5-HT_7 receptor antagonist, methiothepin (Hoyer et al., 1994), significantly inhibited the *S*-(+)-ketoprofen-induced antinociceptive response. The mechanism of such an interaction remains to be elucidated but it could comprise 5-HT release, as previously demonstrated for acetaminophen, which was shown to increase 5-HT levels in the cerebral cortex and pons following acute administration (Pini et al., 1996, 1997a,b), upon release, 5-HT could target specific 5-HT receptors in brain areas implicated in pain processing. Among candidate receptor targets of 5-HT, evidence has been provided for 5-HT_2 receptors in the analgesic effect of acetylsalicylic acid in the hot-plate test in rats (Sandrini et al., 2002a). Interestingly, the preferential cyclo-oxygenase 2 inhibitor, rofecoxib, was shown to increase 5-HT levels and decrease the maximum number of 5-HT_2 receptors in the frontal cortex, this effect was achieved at the dose that produced an analgesic effect in the hot-plate and formalin tests in rats (Sandrini et al., 2002b). Similar results were reported for acetaminophen, which produced a significantly increased tail flick latency and down-regulation of 5-HT_{2A} receptors in the frontal cortex of rats upon acute and 15-day chronic administration (Srikiatkhachorn et al., 1999). Thus, 5-HT release may be a major step in the mechanism underlying the analgesia produced by some NSAIDs. Since methiothepin is also a high-affinity 5-HT_7 receptor antagonist (Terrón, 1998), a role for these receptors cannot be excluded. Indeed, high levels of expression of 5-HT_7 transcripts were detected centrally in areas implicated in sensory and pain processing, including the medial geniculate nucleus, superior and inferior colliculi, central gray and spinal trigeminal nuclei (To et al., 1995).

As far as the effects of tropisetron are concerned, the present observations support a role for spinal 5-HT_3 receptors in the antinociceptive effect of *S*-(+)-ketoprofen. It has been shown in this regard that activation of 5-HT_3 receptors in spinal afferent fib is involved in the transmission of nociceptive information to the central nervous system (Pelissier et al., 1996). With regard to 5-HT_4 receptors, for which tropisetron displays micromolar affinity (Hoyer et al., 1994), the drug concentration administered locally into the spine was 316 μM, a concentration which makes an interaction with 5-HT_4 receptors possible. However, although a role of 5-HT_4 receptors in antinociception through the central amplification of cholinergic transmission has been reported in mice (Ghelardini et al., 1996), no evidence has been provided for the involvement of 5-HT_4 receptors in pain modulation at a spinal level. Further experiments with a selective 5-HT_4 receptor antagonist will be required to determine whether spinal 5-HT_4 receptors play a role in the antinociceptive response to *S*-(+)-ketoprofen in our model.

The present data show an ability of *S*-(+)-ketoprofen to activate serotonergic mechanisms, possibly involving 5-HT release, to yield its antinociceptive effect. Although *S*-(+)-ketoprofen can inhibit prostaglandin synthesis at a central level (Herrero et al., 1997; McCormack, 1994), its interaction with the 5-HT system might explain its higher efficacy with regard to other NSAIDs (Kubota et al., 1979; Bagán et al., 1998; Cabré et al., 1998). From these and other observations, it seems that the analgesic effects of various NSAIDs comprise interactions with the serotonergic system at supra-spinal and spinal levels.

In conclusion, *S*-(+)-ketoprofen exerts antinociceptive effects in the pain-induced functional impairment model in the rat via an interaction with central serotonergic mechanisms, but not with opiate and L-arginine-NO systems. Such

an interaction with the 5-HT system may comprise activation of supra-spinal 5-HT_1/5-HT_2/5-HT_7 receptors and most likely spinal 5-HT_3 receptors. Further experiments using selective 5-HT receptor antagonists will be required to elucidate the involvement of specific 5-HT receptor subtypes in *S*-(+)-ketoprofen-induced antinociception.

Acknowledgements

We wish to thank F. Sánchez, L. Oliva and A. Huerta for their technical assistance. M. Déciga-Campos is a fellow of the National Council for Science and Technology (CONACYT), Mexico.

References

Bagán, J.V., López, A.S., Valencia, E., Santamaría, J., Eguidazu, I., Horas, M., Foros, M., Zapata, A., Artigas, R., Mauleón, D., 1998. Clinical comparison of dexketoprofen tromethiothepinamol and dipyrone in postoperative dental pain. J. Clin. Pharmacol. 38, 55S–64S.

Barbanoj, M., Gich, I., Artigas, R., Tost, D., Moros, C., Antonijoan, R., García, L., Mauleón, D., 1998. Pharmacokinetics of dexketoprofen tromethamol in healthy volunteers after single and repeated oral doses. J. Clin. Pharmacol. 38, 33S–40S.

Bardin, L., Lavarenne, J., Eschalier, A., 2000. Serotonin receptor subtypes involved in the spinal antinociceptive effect of 5-HT in rats. Pain 86, 11–18.

Beltran, J., Martin-Mola, E., Figueroa, M., Granados, J., Sanmarti, R., Artigas, R., Torres, F., Forns, M., Mauleon, D., 1998. Comparison of dexketoprofen trometamol and ketoprofen in the treatment of osteoarthritis of the knee. J. Clin. Pharmacol. 38, 74S–80S.

Björkman, R., 1995. Central antinociceptive effects of non-steroidal anti-inflammatory drugs and paracetamol. Acta Anaesthesiol. Scand. 39 (103), 1–44.

Björkman, R., Hedner, J., Hedner, T., Henning, M., 1990. Central, naloxone-reversible antinociception by diclofenac in the rat. Naunyn-Schmiedeberg's Arch. Pharmacol. 342, 171–176.

Cabré, F., Fernandez, M.F., Calvo, L., Ferrer, X., García, M.L., Mauleón, D., 1998. Analgesic, antiinflammatory and antipyretic effects of *S*(+)-ketoprofen in vivo. J. Clin. Pharmacol. 38, 3S–10S.

Carabaza, A., Cabré, F., Rotllán, E., Gómez, M., Gutiérrez, M., García, L., Mauleón, D., 1996. Stereoselective inhibition of inducible cyclooxygenase by chiral nonsteroidal antiinflammatory drugs. J. Clin. Pharmacol. 36, 505–512.

Caroit, M., Forette, B., Hubault, A., Pasquier, P., 1976. Double-blind study of ketoprofen against a placebo in osteoarthritis of the hip. Scand. J. Rheumatol., Suppl. 1976, 123–127.

Cesselin, F., Laporte, A.M., Miquel, M.C., Bourgoin, S., Hamon, M., 1994. Serotonergic mechanisms of pain control. In: Gebhart, G.F., Hammond, D.L., Jensen, T.S. (Eds.), Progress in Pain Research and Management, Vol. 2. Proc. of the VIIth World Congress on Pain, Seattle, IASP Press, Seattle, WA, USA, pp. 669–695.

Covino, B.G., Dubner, R., Gybels, J., Kosterlitz, H.W., Liebeskind, J.C., Sternbach, R.A., Vyklicky, L., Yamamura, H., Zimmermann, M., 1980. Ethical standards for investigations of experimental pain in animals. Pain 9, 141–143.

Dickenson, A.H., Chapman, V., Green, G.M., 1997. The pharmacology of excitatory and inhibitory amino acid-mediated events in the transmission and modulation of pain in the spinal cord. Gen. Pharmacol. 28, 633–638.

Ezcurdia, M., Cortejoso, F.J., Lanzón, R., Ugalde, F.J., Herruzco, A., Artigas, R., Fernández, F., Torres, F., Mauleón, D., 1998. Comparison of the efficacy and tolerability of dexketoprofen and ketoprofen in the treatment of primary dysmenorrhea. J. Clin. Pharmacol. 38, 65S–73S.

Famaey, J.P., Colinet, E., 1976. A double-blind trial of ketoprofen in the treatment of osteoarthritis of the hip. Rheumatol. Rehabil. 15, 45–49.

Garthwaite, J., 1991. Glutamate, nitric oxide and cell–cell signalling in the nervous system. Trends Neurosci. 14, 60–67.

Garthwaite, J., Charles, S.L., Chess-Williams, R., 1988. Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intracellular messengers in the brain. Nature 336, 385–388.

Ghelardini, C., Galeotti, N., Casamenti, F., Malmberg-Aiello, P., Pepeu, G., Gualtieri, F., Bartolini, A., 1996. Central cholinergic antinociception induced by 5HT4 agonists: BIMU 1 and BIMU 8. Life Sci. 58 (25), 2297–2309.

Herrero, J.F., Parrado, A., Cervero, F., 1997. Central and peripheral actions of the NSAID ketoprofen on spinal cord nociceptive reflexes. Neuropharmacology 36, 1425–1431.

Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R., Humphrey, P., 1994. VII. International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol. Rev. 46, 157–203.

Hunskaar, S., Fasmer, O.B., Hole, K., 1985. Acetylsalicylic acid, paracetamol and morphine inhibit behavioural responses to intrathecally administered substance P or capsaicin. Life Sci. 37, 1835–1841.

Inoue, T., Mashimo, T., Shibata, M., Shibuta, S., Yoshiya, I., 1998. Rapid development of nitric oxide-induced hyperalgesia depends on an alternate to the cGMP-mediated pathway in the rat neuropathic pain model. Brain Res. 792, 263–270.

Kubota, T., Komatsu, H., Kawamoto, H., Yamada, T., 1979. Studies on the effects of anti-inflammatory action of benzoyl-hydrotropic acid (ketoprofen) and others drugs with special reference to prostaglandin synthesis. Arch. Int. Pharmacodyn. 237, 169–176.

Le Bars, D., 1988. Serotonin and pain. In: Osborne, N.N., Hamon, M. (Eds.), Neuronal Serotonin. Wiley, Chichester, pp. 171–229.

López-Muñoz, F.J., Ventura, R., Díaz, I., Fernández-Guasti, A., Tost, D., Cabré, F., Mauleón, D., 1988. Antinociceptive effects of *S*(+)-ketoprofen and other analgesic drugs in a rat model of pain induced by uric acid. J. Clin. Pharmacol. 38, 11S–21S.

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 model). Drug Dev. Res. 28, 169–175.

McCormack, K., 1994. The spinal actions of nonsteroidal anti-inflammatory drugs and the dissociation between their anti-inflammatory and analgesic effects. Drugs 47, 28–45.

Meller, S.T., Dykstra, C., Gebhart, G.F., 1992. Production of endogenous nitric oxide and activation of soluble guanylate cyclase are required for *N*-methyl-D-aspartate-produced facilitation of the nociceptive tail-click reflex. Eur. J. Pharmacol. 214, 93–96.

Netter, P., Lapicque, F., Bannwarth, B., Tamisier, J.N., Thomas, P., Royer, R.J., 1985. Diffusion of intramuscular ketoprofen into the cerebrospinal fluid. Eur. J. Pharmacol. 29, 319–321.

O'Brien, W.M., Grunwaldt, E., 1976. Ketoprofen in the treatment of rheumatoid arthritis: double blind, crossover placebo comparison. Scand. J. Rheumatol. 5 (Suppl. 14), 105–110.

Paxinos, G., Watson, C., 1982. The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.

Pelissier, T., Alloui, A., Caussade, F., Dubray, C., Cloarec, A., Lavarenne, J., Eschalier, A., 1996. Paracetamol exerts a spinal antinociceptive effect involving an indirect interaction with 5-hydroxytryptamine3 receptors: in vivo and in vitro evidence. J. Pharmacol. Exp. Ther. 278 (1), 8–14.

Pini, L.A., Sandrini, M., Vitale, G., 1996. The antinociceptive action of paracetamol is associated with changes in the serotonergic system in the rat brain. Eur. J. Pharmacol. 308, 31–40.

Pini, L.A., Vitale, G., Sandrini, M., 1997a. Serotonin and opiate involve-

ment in the antinociceptive effect of acetylsalicylic acid. Pharmacology 54, 84–91.

Pini, L.A., Vitale, G., Ottani, A., Sandrini, M., 1997b. Naloxone-reversible antinociception by paracetamol in the rat. J. Pharmacol. Exp. Ther. 280, 934–940.

Sacerdote, P., Monza, G., Mantegazza, P., 1985. Diclofenac and pirprofen modify pituitary and hypothalamic beta-endorphin concentrations. Pharmacol. Res. Commun. 17, 679–684.

Sandrini, M., Vitale, G., Pini, L.A., 2002a. Central antinociceptive activity of acetylsalicylic acid is modulated by brain serotonin receptor subtypes. Pharmacology 65 (4), 193–197.

Sandrini, M., Vitale, G., Pini, L.A., 2002b. Effect of rofecoxib on nociception and the serotonin system in the rat brain. Inflamm. Res. 51 (3), 154–159.

Srikiatkhachorn, A., Tarasub, N., Govitrapong, P., 1999. Acetaminophen-induced antinociception via central 5-HT(2A) receptors. Neurochem. Int. 34 (6), 491–498.

Suesa, N., Fernández, M.F., Gutiérrez, M., Rufat, M.J., Rotllán, E., Calvo, L., Mauleón, D., Carganico, G., 1993. Stereoselective cyclooxygenase inhibition in cellular models by the enantiomers of ketoprofen. Chirality 5, 589–595.

Terrón, J.A., 1998. The 5-HT_7 receptor: a target for novel therapeutic avenues? Investig. Drug 1, 302–310.

To, Z.P., Bonhaus, D.W., Eglen, R.M., Jakeman, L.B., 1995. Characterisation and distribution of putative 5-HT_7 receptors in guinea-pig brain. Br. J. Pharmacol. 115, 107–116.

Yaksh, T.L., 1979. Direct evidence that spinal serotonin and noradrenaline terminals mediate the spinal antinociceptive effects of morphine in the periaqueductal gray. Brain Res. 160, 180–185.

Yaksh, T.L., 1997. Pharmacology and mechanisms of opioid analgesic activity. Acta Anaesthesiol. Scand. 41, 94–111.

Yaksh, T.L., Rudy, T.A., 1976. Chronic catheterization of the spinal subarachnoid space. Physiol. Behav. 17, 1031–1036.

Yaksh, T.L., Wilson, P.R., 1979. Spinal serotonin terminal system mediates antinociception. J. Pharmacol. Exp. Ther. 208, 446–453.

Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16, 109–110.