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Evidence against the Participation of μ- and κ-Opioid Receptors in the Analgesic Activity of Ketorolac in Rats

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Abstract

The possibility that activation of opioid receptors is involved in the analgesic activity of ketorolac was explored. The analgesic effects of ketorolac, of ketocyclazocine, the prototype κ-agonist, and of morphine, the prototype μ-agonist, were assayed in the pain-induced functional impairment model in the rat.

All three drugs induced a significant analgesic effect in this model. Naloxone was able to antagonize the effects of ketocyclazocine and morphine. However, the effect of ketorolac was not blocked by naloxone, although a high dose, 3·2 mg kg^{-1}, capable of blocking κ-receptors was used.

It is concluded that activation of μ- or κ-opioid receptors, by either a direct or an indirect mechanism, does not play a role in the analgesic activity of ketorolac.

Ketorolac is a non-steroidal anti-inflammatory analgesic drug which has been demonstrated to be effective in the treatment of moderate to severe pain (Bloomfield et al 1986). Ketorolac has shown an analgesic efficacy comparable with that of opioid drugs in clinical studies (Yee et al 1986; O'Hara et al 1987; Forbes et al 1990) as well as in animal models of experimental pain (Rooks et al 1982; Granados Soto et al 1993), being remarkably more potent than other aspirin-like compounds. It has been demonstrated that ketorolac, as other non-steroidal anti-inflammatory drugs, is able to inhibit prostaglandin synthesis (Rooks et al 1982; Rauk & Laifer 1993). However, due to its high potency and efficacy, it has been suggested that activation of opioid receptors could also be involved in its antinociceptive action (Domer 1990; Uphouse et al 1993).

A direct activation of opioid receptors seems unlikely, since it has been demonstrated that ketorolac is unable to bind to μ-, κ- or δ-opioid receptors (López et al 1987; Yee & Waterbury 1987). Notwithstanding, Domer (1990) proposed an indirect action of ketorolac by inducing the release of endorphins or enkephalins, as this compound was active in opioid-specific assays such as the hot-plate test. That report, however, is not consistent with previous observations showing that ketorolac, as other aspirin-like agents, did not exhibit any significant activity in the hot-plate assay (Rooks et al 1982). Recently, Uphouse et al (1993) suggested that spinal opioid κ-receptors could play a role in the mechanism of action of ketorolac since norbinaltorphimine, a κ-antagonist, was able to block its antinociceptive activity.

The purpose of this study was to explore the participation of opioid receptors in the analgesic effect of ketorolac. We compared the analgesic activity of ketorolac, ketocyclazocine and morphine in the presence and in the absence of the nonspecific opioid-antagonist naloxone. Ketocyclazocine was chosen as it is considered the prototype agonist of the opioid κ-receptor (Martin et al 1976; Vaupel & Cone 1991), morphine being the prototype μ-agonist (Martin et al 1976). Analgesic activity was assessed using the pain-induced functional impairment model in the rat (PIFIR), a procedure which has been shown to be suitable for the assay of both opioid and non-steroidal anti-inflammatory drugs (Granados-Soto et al 1992; López-Muñoz et al 1993).

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Materials and Methods

Animals

Female Wistar rats, 180–220 g, were used in this study. Animals had free access to food and water. All experiments followed the recommendations of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (1980) and the guidelines on ethical standards for investigation of experimental pain in animals (Zimmermann 1983).

Measurement of analgesic activity

Pain was induced and the analgesic effect of ketorolac, ketocyclazocine and morphine was measured by the PIFIR procedure, as described previously (López-Muñoz et al 1993). Rats received an intra-articular injection of 0·05 mL 30% uric acid suspended in mineral oil in the knee joint of the right hind limb under light anaesthesia with ether. Immediately, an electrode was fastened to each hind-paw between the plantar pad. Rats were allowed to recover from anaesthesia and were then placed on a stainless-steel cylinder of 30 cm diameter. The cylinder was rotated at 4 rev min^{-1}, forcing the rats to walk. The variable measured in this method 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. Animals did not show any visible sign of severe discomfort, such as licking, elevating, biting, shaking or vocalization.

After the uric acid injection, rats developed a progressive dysfunction of the injured limb. This was recorded as a diminished time of contact between the right hind-paw and the cylinder. Data are expressed as the functionality index (FI), i.e. the time of contact of the injured right limb divided by the time of contact of the control left limb, multiplied by 100. After approximately 2 h, the FI was zero; the injected limb made no contact with the cylinder. Thus, this time was considered as time zero for measurements of analgesia and rats received the analgesic agent dissolved in saline (0·9% NaCl) as a subcutaneous injection; recordings were carried out for the next 4 h. Recovery of the FI was considered as the expression of the analgesic effect.

Study design

Ketorolac (Syntex, Mexico City) was tested at a dose of 1 mg kg^{-1}. This dose was chosen as it has been previously demonstrated that it yields a significant analgesic effect in the PIFIR model (Granados-Soto et al 1993). Ketocyclazocine (Instituto Miles de Terapéutica Experimental, Mexico City) and morphine (Mexican Secretariat of Health) were tested at a dose of 10 mg kg^{-1}. All analgesic drugs were dissolved in saline and administered subcutaneously to rats previously injured with uric acid. FI was determined at 0, 0·5, 1, 1·5, 2, 2·5, 3, 3·5 and 4 h for ketorolac and morphine. For ketocyclazocine, FI was determined at 0, 0·25, 0·5, 0·75, 1, 1·5, 2, 2·5, 3, 3·5 and 4 h, since its has been reported that the effect of this agent is of shorter duration (Martin et al 1976). FI against time curves were constructed and the maximal observed effect (E_{max}^{obs}) was directly determined from these plots. The area under the effect-against-time curve (AUC_E) was estimated by the trapezoidal rule. Analgesic activity was evaluated using these two pharmacodynamic parameters. E_{max}^{obs} is considered as an indicator of analgesic efficacy and AUC_E as an overall expression of the analgesic action during the whole observation period and hence considering both the intensity and the duration of the effect (Granados-Soto et al 1992; López-Muñoz et al 1993).

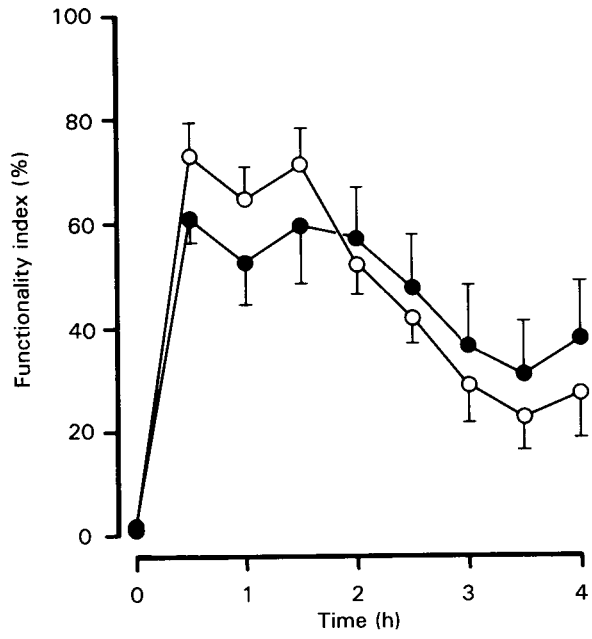

FIG. 1. Time course of the analgesic effect induced by 1 mg kg^{-1} ketorolac, measured as functionality index recovery in rats submitted to pain-induced functional impairment by intra-articular injection of 30% uric acid in the right hind knee in the absence (○) and in the presence (●) of 3·2 mg kg^{-1} naloxone. Data are presented as mean ± s.e.m. of at least eight determinations.

The ability of naloxone (Sigma Chemical Co., St Louis, MO, USA) to block the effects of the three assayed analgesic agents was tested. Naloxone is a nonspecific opioid antagonist, exhibiting a higher affinity for μ-receptors. We therefore assayed morphine, the μ-agonist, in presence of a low (0·1 mg kg^{-1}) naloxone dose. Ketocyclazocine, the κ-agonist, and ketorolac were assayed in the presence of a high (3·2 mg kg^{-1}) naloxone dose. Naloxone was dissolved in saline and injected intraperitoneally 10 min before the administration of the agonists. E_{max}^{obs} and AUC_E were determined for each analgesic agent as described above and compared with the values observed in the absence of naloxone by Student's t-test for unpaired data.

Results

Ketorolac was able to induce a significant analgesic effect in the PIFIR model (Fig. 1). After administration of a dose

Table 1. Analgesic effect of ketorolac (1 mg kg^{-1}), ketocyclazocine (10 mg kg^{-1}) and morphine (10 mg kg^{-1}) in the absence and in the presence of naloxone in the pain-induced functional impairment model in the rat. Analgesia is expressed as the maximal observed recovery of functionality index (E_{max}^{obs}) and the area under the functionality index-against-time curve (AUC_E).

Analgesic agent	n	Naloxone (mg kg^{-1})	E_{max}^{obs} (%)	AUC_E (% h)
Ketorolac	12	—	86·6 ± 4·3	183·9 ± 9·6
	8	3·2	74·1 ± 6·5[a]	181·4 ± 30·5[a]
Ketocyclazocine	6	—	58·9 ± 6·3	27·9 ± 3·0
	6	3·2	4·0 ± 1·3[b]	0·9 ± 0·3[b]
Morphine	6	—	83·4 ± 3·7	186·3 ± 13·0
	6	0·1	10·2 ± 2·5[c]	10·7 ± 3·6[c]

Data are expressed as mean ± s.e.m.; [a] not significantly different from ketorolac alone ($P > 0·01$); [b] significantly different from ketocyclazocine alone ($P < 0·001$); [c] significantly different from morphine alone ($P < 0·0001$).

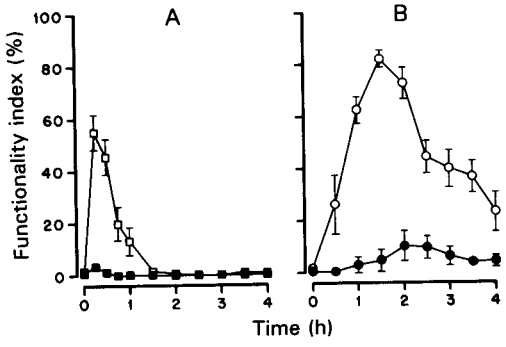

FIG. 2. Time course of the analgesic effect, measured as functionality index recovery in rats submitted to pain-induced functional impairment by intra-articular injection of 30% uric acid in the right hind knee. A. Animals received ketocyclazocine 10 mg kg^{-1} in the absence (□) and in the presence (■) of 3·2 mg kg^{-1} naloxone. B. Animals received morphine in the absence (○) and in the presence (●) of 0·1 mg kg^{-1} naloxone. Data are presented as mean ± s.e.m. of six determinations.

of 1 mg kg^{-1}, FI recovered, reaching a maximum of about 85% and then exhibited a gradual decay. Naloxone (3·2 mg kg^{-1}) did not modify the analgesic profile of ketorolac. There were no significant differences in E_{max}^{obs} nor in AUC_E in the presence and in the absence of the opioid antagonist (Table 1). Ketocyclazocine and morphine were able to induce a significant recovery of the FI, although with a different time course (Fig. 2). Ketocyclazocine exhibited a rapid analgesic effect, reaching a maximal recovery of FI in 15 min. However, the effect was lower than that produced by ketorolac and was transient, FI being not significantly different from zero after 1·5 h. Morphine induced a gradual recovery of the FI which reached its maximum, similar to the E_{max}^{obs} obtained with ketorolac, in 1·5 h; a decrease in the analgesic effect, which was also gradual, was then observed. As a consequence of the difference in duration of the analgesic effect, there was a marked difference in AUC_E between the two opioid drugs. AUC_E values for ketocyclazocine amounted to only approximately 15% of those of morphine, despite the fact that the E_{max}^{obs} of the κ-agonist was about 70% of that of morphine (Table 1). These results clearly demonstrate the usefulness of the estimation of the time course of analgesia, and not only the effect at an arbitrary fixed time, when comparing antinociceptive drugs (López-Muñoz et al 1993). The doses of ketorolac and morphine used in this study appeared to be equi-analgesic, as both E_{max}^{obs} and AUC_E values were similar (Table 1). These data confirm the high analgesic potency of ketorolac.

Unlike the case of ketorolac, naloxone was able to antagonize the effect of the two opioid drugs (Fig. 2). A 3·2 mg kg^{-1} naloxone dose practically abolished the effect of ketocyclazocine, as indicated by the dramatic reduction in both E_{max}^{obs} and AUC_E (Table 1). These results strongly suggest that blockade of κ-receptors was achieved. A lower (0·1 mg kg^{-1}) naloxone dose was able to antagonize the analgesic effect of morphine as indicated by the significant reductions induced in E_{max}^{obs} and AUC_E (Table 1). These results were expected, since naloxone exhibits a higher affinity for μ-receptors.

Discussion

There is disagreement on whether an activation of opioid receptors play a role in the analgesic action of ketorolac. Results reported using opioid-specific assays are contradictory. Rooks et al (1982) did not observe any analgesic activity in the hot-plate test, while Domer (1990) reported that ketorolac exhibits a significant analgesic activity in this assay which could be blocked by naloxone. Recently, Uphouse et al (1993) reported that ketorolac failed to induce analgesia in the hot-plate assay, but was effective in inhibiting the abdominal stretching produced by *p*-phenylquinone injections, the latter effect being blocked by norbinaltorphimine, a κ-antagonist, but not by naloxone. It is clear that ketorolac does not bind to opioid receptors (López et al 1987; Yee & Waterbury 1987). Moreover, ketorolac does not induce tolerance nor precipitate abstinence to morphine (Uphouse et al 1993). Hence it seems unlikely that ketorolac has a direct activity, as either an agonist or an antagonist, on the opioid receptors. However, an indirect action through the release of endogenous peptides, has been proposed (Domer 1990). It has been suggested that the endogenous agonists could be activating κ-receptors at the spinal level (Uphouse et al 1993).

In this work we examined the participation of μ- and κ-opioid receptors in the PIFIR procedure which allows us to follow objectively the time course of analgesia (López-Muñoz et al 1993). Ketocyclazocine and morphine, the prototype agonists of the *k*- and μ-opioid receptors, respectively (Martin et al 1976), were effective in this model, indicating that activation of either receptor subtype can yield analgesia. However, there were differences in the time course of the effect exerted by these agonists. Ketocyclazocine yielded an effect of fast onset which rapidly disappeared. These results are consistent with the observations which have been previously reported in other assays with this κ-agonist (Martin et al 1976; Vaupel & Cone 1991). On the other hand, morphine showed a higher E_{max}^{obs} while the analgesic effect exhibited a gradual onset and lasted longer. Naloxone was able to reduce the effects of both opioid drugs, confirming that this antagonist interacts with both μ- and κ-receptors. To our knowledge, no naloxone-insensitive κ-receptor has been described. The analgesic effect of ketorolac, at either a low or a high dose, was not antagonized by naloxone at a dose level which clearly antagonized the action of ketocyclazocine. Hence, results indicate that an activation, direct or indirect, of κ-receptors does not seem probable. Furthermore, it is also clear that μ-receptors are not involved, as naloxone blocks this receptor subtype at considerably lower doses than those which were ineffective in antagonizing ketorolac. Although Domer (1990) claimed that the analgesic effect of ketorolac could be blocked by naloxone, these observations are inconsistent with those reported by other investigators (Rooks et al 1982; Uphouse et al 1993), as is the case with the results presented here. Moreover, the methodology used by Domer (1990) has been criticized (Uphouse et al 1993).

We have confirmed the remarkable analgesic potency of ketorolac. In a previous study we observed that, in the PIFIR model, ketorolac is even more potent than morphine (Granados-Soto et al 1993). Hence, it seems likely that this

high potency cannot be solely explained by inhibition of prostaglandin synthesis, but also by other mechanisms of action. The present results provide evidence that such additional mechanisms do not include activation of μ- or κ-opioid receptors, either directly or by participation of endogenous opioids. It has been reported that ketorolac exhibits iontophoretic-like properties and it has been suggested that such characteristics may contribute to its analgesic activity (Chávez et al 1993). Recently, evidence has been provided for a central mechanism of ketorolac, in addition to its peripheral action (Malmberg & Yaksh 1993; Uphouse et al 1993). The observations of an antagonism of ketorolac by intrathecal administration of norbinaltorphimine (Uphouse et al 1993) can be explained by a non-opioid mechanism, as this compound is able to antagonize cannabinoid-induced antinociception in addition to its κ-blocking properties (Welch 1993). It has been shown that naloxone fails to block the antinociceptive action of cannabinoids (Welch & Stevens 1992; Welch 1993), as we have shown is the case for ketorolac. The possibility that ketorolac interacts with cannabinoid sites in the central nervous system warrants further investigation.

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