Piroxicam-induced analgesia: Evidence for a central component which is not opioid mediated

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Abstract. Piroxicam is a nonsteroidal anti-inflammatory drug with a potent analgesic effect. In order to establish whether the analgesic action of Piroxicam has a central component, we studied the effect of the drug on the nociceptive orbicularis oculi reflexes evoked by electrical stimulation of the cornea and supraorbital nerve in healthy subjects. Piroxicam significantly suppressed the corneal reflex and R3 component of the blink reflex by 28% ($p < 0.05$) and 50% ($p < 0.01$), respectively. This effect was not reversed by the i.v. injection of naloxone. Beta-endorphin levels did not change. Piroxicam administration induces distinct inhibitory changes in nociceptive reflexes, which suggests that the analgesic action of the drug has a central component. The ineffectiveness of naloxone, and the lack of beta-endorphin changes, indicate that this central action is independent of the opioid system; other pain regulatory systems are probably involved.
Key words. Piroxicam; analgesia; opioid peptides.

Analgesic agents are frequently divided into two groups of drugs which can be classified as acting centrally or peripherally. The analgesic mechanism of piroxicam, a non steroidal anti-inflammatory drug (NSAID), has been related to inhibition of the cyclo-oxygenase enzyme and to prevention of the conversion of the arachidonic acid to the cyclic endoperoxides[1,2]. Interestingly, it has recently been shown that other NSAIDs, such as diclofenac, ketoprofen and pirprofen, also exert a central action through the activation of the endogenous opioid system[3,4]. Since piroxicam has been shown to induce a profound and long-lasting analgesic effect, which sometimes occurs without a direct correlation with the improvement of the local inflammatory reaction[5,6], this drug might also rely on a central mechanism of action. To verify this hypothesis, we studied the effect of piroxicam on the nociceptive orbicularis oculi reflexes in normal volunteers[7]. The effect of naloxone administration and plasma beta-endorphin levels were also measured, to evaluate a possible involvement of the opioid system in the drug-induced analgesia.

Material and methods

Subjects. Six healthy volunteers (4 males and 2 females), aged 25–38 years, entered the study. All subjects gave consent and the research was approved by the local Ethical Committee.

Experimental procedure. Subjects participated in a crossover single blind study with two weekly sessions: piroxicam and saline. In each session an intravenous cannula was inserted at 08.00 h and blood was withdrawn every 15 min for 30 min before the injection of 40 mg i.m. piroxicam (Feldene, Pfizer) or saline and thereafter at 30, 60, 90 and 120 min after injection. At the last time 2 mg naloxone were administered i.v. and another blood sample was taken after 5 min. Plasma was separated by centrifugation and immediately frozen at $-70\,^{\circ}C$ until beta-endorphin assay was performed as previously described[8]. Briefly, samples were analyzed by RIA after concentration and extraction through Sep-pak cartridges with a final recovery of 70% using a purchased beta-endorphin kit (NEN, Still Water, Minnesota) with an assay sensitivity of 1 pg/tube. The intra- and interassay coefficients of variation were 6 and 10%, respectively.

At the same times as blood samples were taken, blink and corneal reflexes (fig. 1) were evoked by electrical stimulation of the supraorbital nerve and cornea, respectively, as previously described[9,10]. Briefly, square-wave negative single pulses of 0.1 ms/10–80 mA (blink) and 1 ms/0.2–3 mA (corneal) were delivered arhythmically at a low rate of 2–5 pulses/min. For the blink reflex the stimulus strength was adjusted to approximately 10 times the sensory threshold, while for the corneal reflex the stimulus intensity was increased to approximately 4 times the sensory threshold. These intensities evoked maximal reflex responses, and induced painful sensations which were strong, but that the subject could nevertheless bear for the whole session. Electromyographic signals were recorded from the orbicularis oculi muscle through surface electrodes; signals were rectified and averaged (12 trials); the area of averaged responses was automatically measured.

Statistical analysis. All measures were normalized as percentages of the values obtained in the series preceding the administration of piroxicam or saline. Intraindividual differences between series within a session were evaluated by Student's t-test for paired data. Group differences between sessions, as well as differences between different reflexes, were evaluated by analysis of variance (F-test).

Results

Figure 1 shows the reflex responses recorded in the piroxicam session in a representative subject, and figure 2 summarizes quantitative results in the whole group of subjects. R1 and R2 were not affected, whereas the corneal reflex and R3 were suppressed by piroxicam. Maximal reductions were attained after 60 min for both the corneal reflex ($-28.5\% \pm 2.74$ SD, $p < 0.05$ vs

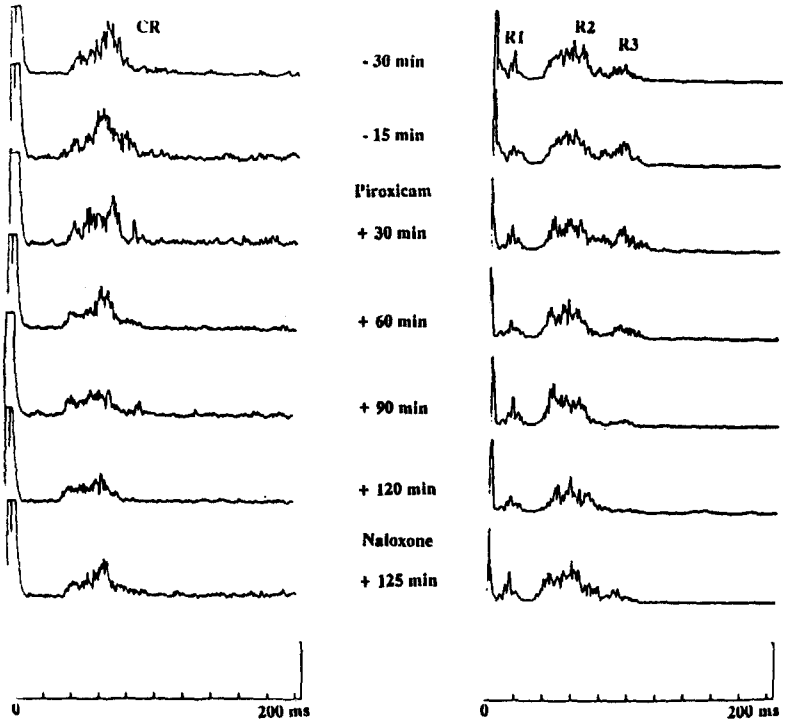

Figure 1. Recording of the orbicularis oculi reflexes in the piroxicam session. Surface recordings from the orbicularis oculi muscle in one subject. On the left, corneal reflex (CR) evoked by electrical stimulation of the cornea. Rectified and averaged signals; vertical calibration: 500 μV. On the right, blink reflex with its three components (R 1, R 2 and R 3) evoked by electrical stimulation of the ipsilateral supraorbital nerve.

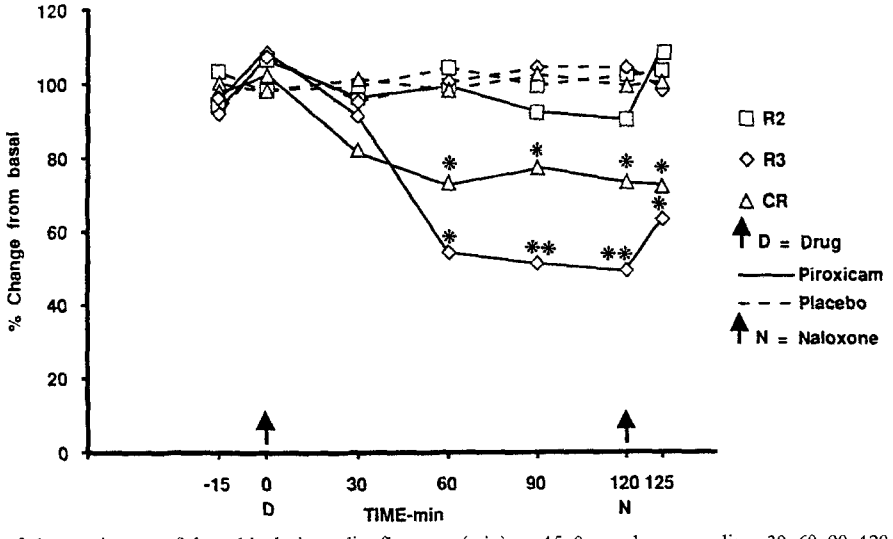

Figure 2. Line graphs of changes in area of the orbicularis oculi reflexes in the piroxicam (solid lines) and placebo (dashed lines) sessions. Y-axis: mean area of responses expressed as % of baseline values. X-axis: time (min): − 15, 0, pre-drug recording; 30, 60, 90, 120, post-drug recording; 125, post-naloxone recording. * $p < 0.05$ and ** $p < 0.01$ vs placebo values.

placebo) and R 3 (− 50.4% ± 4.7 SD, $p < 0.01$ vs placebo); thereafter these changes persisted throughout the experimental session, and were not modified by the i.v. injection of naloxone. Placebo did not cause any significant variation of the reflex responses tested. In all subjects, neither piroxicam nor placebo administration changed plasma beta-endorphin levels (table).

Discussion

Recording of the orbicularis oculi reflexes allows one to differentiate, to some extent, the muscle-relaxant, sedative, and analgesic properties of central acting neurotropic drugs [7].

The components of the blink reflex (R 1, R 2 and R 3) and the corneal reflex share the same motoneurons, but differ

Plasma beta-endorphin levels (mean ± SD) in the six subjects studied before and after 40 mg i.m. piroxicam or saline

Time (min)	Piroxicam (fmoles/ml)	Saline
− 15	3.9 ± 1.2	3.7 ± 0.9
0	3.5 ± 0.9	3.4 ± 0.6
30	3.4 ± 0.5	3.4 ± 0.4
60	4.5 ± 1.2	3.7 ± 0.5
90	4.6 ± 0.6	3.6 ± 0.8
120	3.6 ± 0.5	3.5 ± 0.3
(N) 125	4.2 ± 1.1	3.6 ± 0.6

(N), naloxone administration.

in the afferent fibers and central circuits. R 1 is mediated by Aβ cutaneous afferents and a short pontine circuit, and is relatively independent from cortical and reticular influences[11,12]. R 1 is not affected by the potent narcotic analgesic fentanyl and is affected little by diazepam[7,12,13], possibly by the muscle-relaxant action of this drug[7,14]. R 2 is also mediated by Aβ cutaneous afferents and a polysynaptic chain of interneurons in the pontomedullary lateral reticular formation[10,11]; it is highly susceptible to cortical and reticular influences, is affected little or not at all by fentanyl[7,12,13], but is diminished by nicotine[15] and baclofen (a GABA-b agonist), and strongly suppressed by diazepam[7,12,13]. This suppression by CNS depressants is mainly related to a sedative action through cortical and reticular receptors[7,13]. R 3 is evoked by painful high-intensity shocks, but little is known about its physiology[17]. Finally, the corneal reflex is a purely nociceptive reflex. The cornea is exclusively innervated by small myelinated fibers (Aδ) and unmyelinated fibers, which convey pain sensations, and project to nociceptive specific and wide-dynamic-range neurons of the spinal trigeminal nucleus and pars caudalis[7,18]. The corneal reflex is transmitted through fewer synapses than R 2 and is more resistant to cortical-reticular perturbations[9]. In an earlier study[7], we found that 1.5 mg i.m. fentanyl – the same dosage that had little or no effect on R 1 and R 2 – suppressed the corneal reflex by 70% and R 3 by 90%, and these changes were completely reversed by the specific antagonist naloxone.

In the present study, piroxicam affected neither R 1 nor R 2, which indicates that no muscle-relaxant or sedative effects were exerted. Conversely, the corneal reflex and R 3 were significantly suppressed by 28% and 50%, respectively. Piroxicam, therefore, inhibits the nociceptive reflexes as fentanyl does, though to a lower degree. This finding demonstrates a central analgesic action, because the electrical stimuli – used in this study to elicit the reflexes – directly excite the nerve fibers, by-passing the receptor. The known peripheral action that piroxicam might exert is therefore without influence in our model. Finally, the central analgesic action of piroxicam appears to be independent of the opioid system, since naloxone did not reverse the reflex changes and beta-endorphin levels were unmodified throughout the recording session.

Although no other NSAIDs have been evaluated with our experimental procedure, it has been shown by means of other methods that indomethacin, diclofenac, aspirin, ketoprofen and ibuprofen, besides having a peripheral action, may have a central effect in depressing pain sensation[19–22]. Indomethacin and diclofenac administered by intracerebroventricular injection inhibited nociceptive responses in arthritic rats[19]. In human volunteers, acetylsalicylic acid reduced the late pain-related response in the cortical potential evoked by electrical stimulation of the tooth[20], and ketoprofen elevated the threshold of nociceptive reflex activity elicited in the femoral biceps muscle by electrical stimulation of the sural nerve[21]. The latter effect could not be replicated in paraplegic patients, which indicates a supraspinal involvement in the central analgesic effect of the drug. Finally, intravenous injection of indomethacin, ibuprofen and diclofenac is capable of depressing the neural activity in the dorsomedial part of the neutral nucleus of the rat thalamus elicited by electrical stimulation of afferent C fibers in the sural nerve[22].

Regarding the mechanism of action of NSAIDs in inducing central analgesia, there are observations which indicate the involvement of opioid and non-opioid pain-related pathways. Sacerdote et al.[4] clearly showed that chronic treatment with two cyclooxygenase inhibitors, such as diclofenac and pirprofen, increases the hypothalamic content and pituitary secretion of beta-endorphin in rats. On the other hand Paeile et al.[6] found that i.v. indoprofen, another NSAID, has a potent effect on the bioelectric activity of the cortex, and increases evoked cortical potentials in a manner similar to that induced by the administration of morphine. However, in contrast to opiates it is not antagonized by naloxone. Our findings showed that piroxicam administration did not modify beta-endorphin levels and that the changes in nociceptive reflexes were naloxone-insensitive; these observations suggest that the central effect of the drug is not related to the activation of the opioid receptor.

Marrazzi et al.[23] reported that the intracarotid injection of prostaglandin $F_{2\alpha}$ provokes a reduction of evoked potentials; thus it is possible to postulate that inhibition of central prostaglandin synthesis may be involved in the central action of piroxicam, as is known to occur for the peripheral sites[2]. An alternative hypothesis is to postulate the involvement of serotoninergic mechanisms. Electrophysiological data in adult rats showed that thalamic firing evoked by noxious stimuli could be totally blunted by i.v. administration of acetyl salicylate of lysine, and that this effect could be counteracted by pretreating the animals with metergoline, a serotonin receptor antagonist, while naloxone had no effect[24].

In conclusion, our results provide evidence for a central component in the mechanism of action of piroxicam, and exclude that this is due to an activation of the endogenous opioid pathway. Further studies are needed to clarify whether the central effects of piroxicam are mediated

by an inhibition of prostaglandin synthesis and/or by an activation of non-opioid antinociceptive mechanisms such as the serotoninergic pathway.

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Protection by chlorpromazine against lethality and renal toxicity of cisplatin in mice*

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Abstract. The effect of chlorpromazine on acute lethal toxicity and nephrotoxicity induced by cisplatin was studied in mice. Chlorpromazine given (i.p.) 1 h before cisplatin greatly reduced lethal and renal toxicities of cisplatin. Chlorpromazine did not reduce the antitumor activity of cisplatin against Sarcoma 180 in ddY mice or EL-4 Leukemia in C57BL/6J mice.
Key words. Cisplatin; chlorpromazine; lethality; renal toxicity; mice.

cis-Diamminedichloroplatinum (cisplatin; CDDP) is one of the most effective anticancer chemotherapeutic agents used in clinical practice. Its nephrotoxicity is well known as the most important dose-limiting factor[1]. We have been interested in the combined use of routine drugs (e.g., sodium thiosulfate, caffeine, etc.) and CDDP[2,3]. A widely used antiemetic agent, chlorpromazine (CPZ), and related phenothiazines, are routinely used in combination with CDDP to reduce nausea and improve patient compliance[4]. Studies conducted on mice have shown that CPZ offered protection against irreversible renal toxicity produced by nitrosourea and methyl CCNU[5]. The protection mechanism of CPZ is not known. However, it is possible that CPZ could ameliorate some of the more serious side effects of other antitumor agents, including the nephrotoxicity of CDDP. In the following study, we evaluated the possible protective effects of CPZ against CDDP-induced toxicity in mice.

Materials and methods

Male ddY mice weighing 23–25 g and male C57BL/6J mice weighing 22–24 g, obtained from Japan SLC (Hamamatsu), were used. Cisplatin (CDDP) for injection, 10 mg/vial (Briplatin) and chlorpromazine (CPZ) for injection, 25 mg/vial (Wintamine) were purchased from Bristol Myers Co., Tokyo, and Shionogi Pharmaceutical Co., Osaka, respectively.

Acute lethality was recorded over the 8 days following injection. Blood samples were obtained from the orbital vein in heparinized microhematocrit capillary tubes[6]; urea nitrogen levels in serum (BUN) were determined using a kit from Wako Pure Chem. Co., Tokyo. In preliminary experiments, we established that BUN levels were maximally elevated on day 4 following CDDP treatment and we therefore used this time point for BUN determinations in all subsequent experiments. To assess the effect of CPZ on the antitumor activity of CDDP,