Opioids are given under general anaesthesia to provide adequate intraoperative analgesia but also to reduce the requirements of anaesthetics and thus reduce their side-effects. Other analgesics are also given to potentiate the analgesic effect such as cyclooxygenase (COX) inhibitors, most of them also known as non-steroidal anti-inflammatory drugs (NSAIDs). However, the potential interference with opioids to reduce halogenated anaesthetic requirements remains unclear and contradictory experimental results in animals have been reported.1, 2

The relative potency of analgesic drugs during inhalation anaesthesia has been determined through their reduction of the minimum alveolar concentration (MAC) of halogenated anaesthetics3, 4 where a typical dose–response effect has been observed.5 A previous study in rats2 determined a potentiation of the effect of morphine on the MAC of isoflurane by aspirin, a covalent modifier of COX-1, where only one-third of the morphine dose was necessary, when combined with the NSAID, to reduce the MAC of isoflurane to a similar degree. Since aspirin alone did not provide any sparing effect on isoflurane requirements, a synergistic effect was determined. However, a similar study, where the preferential COX-2 meloxicam was used, failed to demonstrate a synergistic or additive effect on the MAC of isoflurane.1 The observed difference might be a consequence of the differential action on COX receptors. There are no
studies on the interaction of opioids and COX inhibitors in anaesthetized humans, although such interactions have been reported in domestic animals.5–8

Reasons to improve intraoperative analgesia when giving opioids also include the prevention of acute opioid tolerance, which would decrease postoperative, and probably intraoperative, analgesic potency.9 Tolerance to opioids might be a consequence of direct opioid activation of pronociceptive pathways10 involving the release of cytokines and COX11 12 among other mediators, which might be prevented by administration of COX inhibitors. We hypothesized that COX inhibitors may differently potentiate opioids to reduce the MAC value during inhalation anaesthesia and also prevent opioid tolerance. The aim of this study was to determine a differential effect of four clinically used COX inhibitors with variable COX selectivity to allow the selection of those that may provide the highest level of interaction with remifentanil in terms of MAC reduction of sevoflurane in the rat.

**Methods**

After obtaining Institutional Animal Care Committee approval (Madrid, Spain), the reduction in the sevoflurane MAC in response to remifentanil given in continuous infusion was evaluated in rats.

Seventy-two male Wistar rats (Charles River Laboratories, Barcelona, Spain) weighing 370 g (so, 59 g), were housed in groups of four to six, with a 12 h light–12 h dark cycle, relative humidity of 50–70%, and 20 (2)°C room temperature. Food (B&K Universal, Grimston, UK) and water were provided ad libitum. Animals were allowed to acclimatize for at least 1 week. All studies were performed during the morning (starting at 9:00 a.m.).

Rats were placed in an induction chamber into which 8% sevoflurane (Sevorane; Abbott Laboratories, Madrid, Spain) in a continuous oxygen flow of 3 litre min⁻¹ was administered (Sevoflurane Vaporizer, Sevorane Dragèr Vapor 2000). After 2–3 min, the inhaled sevoflurane concentration was reduced to 3–5%. Tracheal intubation was performed using a 14 G polyethylene catheter (Terumo Surflo IV Catheter) with the animal positioned in sternal recumbency. A flexible, blunt-tip guidewire was inserted into the trachea with an infusion pump (Syringe pump, model Sep11S, Ascor S.A., Medical Equipment, Warsaw, Poland) by means of a 22 G polyethylene catheter inserted into a tail vein.

**Monitoring**

The carotid artery was catheterized (Venocath-18, Venisystems, Abbott, Ireland) via surgical cut-down. This access allowed arterial blood sampling and arterial pressure measurement via a calibrated pressure transducer (Transpac IV; Abbott Laboratories, Abbott Park, IL, USA). Systolic, diastolic, and mean arterial pressures, arterial oxygen hemoglobin saturation (via pulse oximetry), and heart rate and ventilatory frequency were continuously monitored and recorded immediately before each MAC step and after 30 min of drug administration (RGB Medical Devices, Madrid, Spain). Arterial blood (1 ml) was collected for blood gas analysis (Rapidlab 860, Bayer AG, Leverkusen, Germany) at the end of study, to ensure that values (at that time point) were within normal limits of pH (7.34–7.44) and oxygen (Pao2; >90 mm Hg) and carbon dioxide arterial partial pressures below 55 mm Hg (Paco2; 35–55 mm Hg). Rectal temperature was also monitored and maintained between 37.0 and 38.5°C by means of a water-circulating warming blanket (Heat Therapy Pump, Model TP-220; Gaymar, Orchard Park, NY, USA) and a heating light.

**Determination of the MAC**

Intratracheal gas sampling was used to measure anaesthetic gas concentration and to determine the MAC. This method has been described in detail previously.13 In brief, a fine catheter with a 0.9 mm external diameter was inserted through the tracheal catheter, with the fine catheter tip located at the level of the carina. The proximal end of the catheter was connected to a 10 ml gas-tight glass syringe (Hamilton Syringe, Sigma-Aldrich, St Quentin Fallavier, France). Samples were obtained by withdrawing 10 ml gas over 5 min using an infusion pump (Mod. 55–2226; Harvard Apparatus, Millis, MA, USA). The fine catheter was withdrawn between samples. After every step change in anaesthetic concentration delivered by the anaesthetic circuit, at least 10 min were allowed for equilibration before obtaining gas samples. The samples were assayed using a side-stream infrared analyzer (Capnomac Ultima, Datex-Ohmeda, Hertfordshire, UK).

The MAC of sevoflurane and of sevoflurane plus remifentanil at a constant rate of infusion was established.14 A painful noxious stimulus was applied with a long haemostat (8 in. Rochester Dean Hemostatic Forceps) clamped to the first ratchet lock onto the tail for 60 s immediately after the gas sample was obtained from the trachea. The tail was always stimulated proximal to a previous test site when the previous response was negative or distal if it was positive, starting 6 cm distal to the tail base. A positive response was considered to be a gross purposeful movement of the head, extremities, or body. A negative response was considered to be the lack of movement or grimacing, swallowing, chewing, or tail flick. Where a negative response was seen, the sevoflurane concentration was then reduced in decrements of 0.20% until the negative response became positive. Similarly, where a positive response was seen, the sevoflurane concentration was then increased until the positive
response became negative. The MAC was considered to be the concentration mid-way between the highest concentration that permitted movement in response to the stimulus and the lowest concentration that prevented such movement. The determination of the MAC was performed in a laboratory 600 m above sea level.

**Experimental design**

The MAC was determined four times in every animal. Once the animals were anaesthetized and instrumented, a baseline MAC was determined, and each animal acted as its own control. Remifentanil was given i.v. at a constant rate of infusion of 120 μg kg⁻¹ h⁻¹ in the tail vein with no loading dose and the MAC was determined then (RMF-1) and again ∼90 min later (RMF-2). Finally, the remifentanil infusion was stopped and the MAC redetermined (post-RMF). Thirty minutes were allowed between MAC determinations and 1 h was usually necessary to determine the MAC value. Overall, every experiment lasted between 6 and 7 h. The animals were randomly allocated to one of two administration groups in which the COX inhibitor was given i.p. either at the same time as the remifentanil infusion was started, immediately after determining baseline MAC (simultaneous administration), or immediately after first determining the MAC of remifentanil (RMF-1, delayed administration). Thirty minutes were allowed between drug administration and after MAC determination. Animals were killed with potassium chloride given i.v. while still deeply anaesthetized at the end of the study. A schematic view of the experimental design is shown in Figure 1.

**Drug groups**

Four COX inhibitors were studied: paracetamol (Paracetamol, Perfalgan, Bristol–Myers Squibb, München, Germany), metamizole (Metamizol; Nolotil, Boehringer Ingelheim, San Cugat del Vallés, Spain), ketoprofen (Ketofen, Merial, Lyon, France), and parecoxib (Parecoxib, Dynastat, Pfizer, Alcobendas, Spain). A pilot study was performed by selecting three doses (low, medium, and high; 1 ×, 2 ×, and 4 ×, respectively) from the clinical dose range for rats of the studied COX inhibitors. The clinical dose range previously reported was 50–300 mg kg⁻¹ for paracetamol, 1–5 mg kg⁻¹ for ketoprofen, and 10–30 mg kg⁻¹ for parecoxib. Reported effective doses for metamizole may greatly differ depending on whether one is referring to its anti-inflammatory or antinociceptive activity and doses of 25–600 mg kg⁻¹ have been studied; doses of 15–60 mg kg⁻¹ were included in the pilot study. These drugs and doses were tested aiming to select the dose providing the highest level of potentiation of remifentanil in decreasing the sevoflurane MAC. When two doses gave similar results, the lowest dose was selected. Studied doses were paracetamol (75, 150, and 300 mg kg⁻¹), ketoprofen (1, 2, and 4 mg kg⁻¹), and parecoxib (7, 15, and 30 mg kg⁻¹). However, since a relatively high range for metamizole doses was found for the rat, the lower doses were administered in the pilot study (15, 30, and 60 mg kg⁻¹).

The selected dose from each COX inhibitor was paracetamol 300 mg kg⁻¹, metamizole 15 mg kg⁻¹, ketoprofen 2 mg kg⁻¹, and parecoxib 30 mg kg⁻¹. Rats were randomly allocated to one of the two administration groups described above, with an n value of eight rats per group. Since a mean remifentanil MAC reduction of 24% was expected and the MAC method involves a normal variation between 5% and 10%, those rats with an MAC reduction of <10% with remifentanil were excluded from the study as non-respondent rats. One further group was given remifentanil plus saline and served as a control (control group). All groups received the remifentanil infusion.
Statistical analysis
Sample size calculation was determined based on the previous data and on a sample size software (N Query Advisor 2.0, Statistical Solutions, Saugus, MA, USA). Animals were randomly assigned to the different groups using a random number generator (Excel 2007, Microsoft Office). Data are presented as mean (SD) and were assessed for normality (Kolmogorov–Smirnov's test) before applying statistical parametric tests. Then, to examine the effect of remifentanil and the studied COX inhibitors over time on MAC, an analysis of variance (ANOVA) for repeated measurements was conducted (by comparison of the percentage of reduction of the MAC; baseline=100%). To compare differences between drug treatments at the two defined study times (RMF-1 and RMF-2), a one-way ANOVA (GLM-UNIANOVA model) was used. The Bonferroni test was used to determine significant differences between different NSAIDs. Finally, a paired-samples T-test was used to determine differences between two study times within a single COX inhibitor or control. A P-value of <0.05 was set to indicate statistical significance. All analyses were performed using standard statistical software (SPSS vs 15 for Windows, SPSS Inc., Wacker Drive, Chicago, IL, USA).

Results
Baseline sevoflurane MAC in rats was similar in all groups with an average value of 2.4 (0.3) vol% (range 1.6–2.9 vol%, n=72). When a remifentanil infusion was given at 120 μg kg⁻¹ h⁻¹ (COX inhibitors were given afterwards; delayed and control, time RMF-1), the mean MAC value was reduced to 1.8 (0.3) vol% (range 1.2–2.5 vol%, n=40, P<0.05) or a 24 (7)% reduction (range 9–42%, n=40, P<0.05).

COX inhibitors differentially potentiate the MAC reduction produced by remifentanil
Among the four studied drugs, only paracetamol was able to produce a significant potentiation of the MAC reduction produced by remifentanil (ANOVA for repeated measures, Bonferroni’s test: delayed administration, P<0.001; simultaneous administration, P=0.002). The maximum MAC reduction was observed at time RMF-2 when paracetamol was given after the determination of the MAC reduction produced by remifentanil [35 (10)%; one-way ANOVA, Bonferroni’s test, P=0.000 vs control] and at time RMF-1 when paracetamol was given simultaneously [34 (4)%; one-way ANOVA, Bonferroni’s test, P=0.021 vs control]. Metamizole, ketoprofen, and parecoxib did not produce a similar effect, suggesting that the level of potentiation of the MAC reduction produced by remifentanil is highly dependent on the considered drug (Table 1 and Fig. 2).

Remifentanil-induced tolerance may be partially prevented by NSAID administration
Remifentanil induced a tolerance effect as evidenced by a reduction in the MAC value within the short time in the control group (RMF-1 vs RMF-2, P<0.001). When any of the

| Table 1 | Baseline MAC of sevoflurane, after a remifentanil constant infusion rate at two times (RMF -1 and RMF -2), and after stopping the remifentanil infusion (post-RMF). Animals were given paracetamol, metamizole, ketoprofen, or parecoxib i.p. when the remifentanil infusion was started (simultaneous) or after the MAC of sevoflurane was determined with the remifentanil infusion (delayed). A control group was given only the remifentanil infusion. Data are expressed as mean (SD). *Different from baseline, same drug. Significant at a P-value of <0.05. n=8/group |
|---------|---------------------------------|----------------|----------------|
| Time    | Control                         | Delayed         | Simultaneous   |
|         | Paracetamol                     | Paracetamol     | Paracetamol    |
|         | Metamizole                      | Metamizole      | Metamizole     |
|         | Ketoprofen                      | Ketoprofen      | Ketoprofen     |
|         | Parecoxib                       | Parecoxib       | Parecoxib      |
| Baseline| 2.4 (0.2)                       | 2.5 (0.3)       | 2.4 (0.2)      |
| RMF-1   | 1.8 (0.2)*                      | 2.0 (0.1)*      | 1.8 (0.2)*     |
| RMF-2   | 2.1 (0.3)                       | 1.9 (0.3)*      | 2.3 (0.3)      |
| Post-RMF| 2.3 (0.3)                       | 2.3 (0.1)       | 2.3 (0.1)      |
Four studied drugs were given after the determination of the MAC reduction produced by remifentanil (Fig. 2), such tolerance effect was not observed (metamizole, ketoprofen, and parecoxib, T-test for paired samples, \( P < 0.05 \) vs control) or was even above that previously observed with remifentanil alone, determining a potentiation of the MAC reduction while preventing opioid tolerance (paracetamol, T-test for paired samples, \( P < 0.000 \)). However, a tolerance effect was observed when the remifentanil infusion was started simultaneously with the administration of the drugs (control and drug groups, T-test for paired samples, RMF-1 vs RMF-2, \( P < 0.05 \)).

Discussion

The reduction of the MAC of sevoflurane produced by remifentanil may be potentiated by the administration of drugs with COX inhibitory action. However, these drugs may greatly vary in their ability to potentiate such reduction, suggesting that the most appropriate combinations should be considered in terms of MAC reduction and, probably, net analgesic effect. Among the studied COX inhibitors, only paracetamol further reduced the MAC with remifentanil, whereas metamizole, ketoprofen, and parecoxib failed to produce such potentiation.

The effects of opioids on MAC have been used to determine their relative analgesic potency. In rats, remifentanil reduces the MAC of isoflurane between 10% and 60% with doses ranging from 60 to 240 \( \mu \text{g} \text{kg}^{-1} \text{h}^{-1} \). Therefore, an MAC reduction of 24% obtained with remifentanil at 120 \( \mu \text{g} \text{kg}^{-1} \text{h}^{-1} \) is similar to that previously reported (25%), although with great inter-individual variation. The differential interaction between remifentanil and the studied COX inhibitors agrees with previous reports where a supra-additive

Fig 2 Reduction of the MAC of sevoflurane at different times produced by remifentanil alone (control) or combined with paracetamol, metamizole, ketoprofen, and parecoxib given at two different time points (top, delayed administration; bottom, simultaneous administration; Fig. 1). Data are expressed as mean (SD), \( n = 8 \). *Different from control group (repeated measures). ‡RMF-2 different from time RMF-1, same drug. ‡‡RMF-2 different from time RMF-1, same drug, in all drug groups. Significant at a \( P \)-value of < 0.05.
Interaction of remifentanil and COX inhibitors in MAC

Interaction has been observed between morphine and aspirin, whereas a lack of interaction has been reported when the NSAID, meloxicam, was used in rats. Similar studies performed in other animal species reported no potentiation of the morphine sparing effect with flunixin meglumine, a COX-1 preferential NSAID commonly used in veterinary practice, in goats or that of butorphanol with meloxicam in rabbits or carprofen in dogs. The magnitude of the potentiation produced by COX inhibitors when added to remifentanil ranged from 30% to 50% relative increase in dogs and rabbits, up to 70–80% relative increase in rats given morphine and aspirin. The observed interaction might be produced at a spinal level, where both opioids and COX inhibitors act and may partly explain its effects on the MAC, since a significant component of the MAC occurs at the spinal cord.

However, a limitation of the study is that extrapolation of the results to humans should be considered with caution. Relevant species differences have been observed with a variable analgesic or tranquilizing action of opioids. Therefore, humans and non-human primates are highly sensitive to the effects of opioids, whereas their effects in horses and pigs are less pronounced. These differences have also been observed in their capacity to reduce the MAC of halogenated anaesthetics; morphine 2 mg kg\(^{-1}\) reduced the MAC of isoflurane by 50–55% in dogs and rhesus monkeys but only by 13% in pigs. Since plasma remifentanil was not measured, there is no assurance that the observed effects were not affected by fluctuations in plasma remifentanil during the study. Also, pharmacokinetics differ from rats to humans with a much higher clearance level in the former species.

Potentiation by COX inhibitors of the remifentanil reduction of sevoflurane MAC might be based on the inhibitory action of the different COX isoenzymes, although other mechanisms cannot be ruled out. Among other potential mechanisms, a synaptic interaction between opioids and NSAIDs has been determined, in which activation of the microreceptor causes presynaptic inhibition of the \(\gamma\)-aminobutyric acid transmitter release mediated by arachidonic acid metabolites. Variable synergism between COX inhibitors and morphine antinociception has been determined in mice at the spinal level in a model of visceral pain where apparently preferential COX-1 inhibitors and COX-2 selective inhibitors showed a similar range of synergistic interaction with the opioid. However, when a mechanical nociceptive stimulus, like the supramaximal stimulus used to determine the MAC, was used, no effect was observed as a consequence of the potential interaction between COX inhibition and opioid action.

ketoprofen is a non-specific COX inhibitor in contrast to the preferential COX-2 inhibitor parecoxib, a prodrug of valdecoxib. Paracetamol and metamizole are analgesic and antihyperthermic agents lacking an anti-inflammatory activity. Recently, it has been demonstrated a potent inhibitory effect on COX-2 by paracetamol in humans with a moderate inhibition of COX-1, with a pharmacological profile similar to that of selective COX-2 inhibitors. A similar action has not been reported for metamizole. Therefore, a selective inhibition on COX-1 and COX-2 does not fully explain the observed effects since meloxicam, a COX-2 inhibitor, failed to produce the same effect in rats but not in dogs. Hence, other potential mechanisms should be considered, despite potential species variation in the action of COX inhibitors. Analogous and antipyretic, but not anti-inflammatory, effects of paracetamol have been attributed to the activation of a COX-3 isoenzyme, also known as COX-1b. However, great differences have been found between the COX-3 isoenzyme in dogs and the isoenzyme as identified in mice or humans, so the COX inhibition produced by paracetamol remains unclear. Among other potential mechanisms, serotonin (5-HT) and cannabinoid receptor activation, but also nitric oxide levels, might be involved in the potentiation of paracetamol in the potentiation of remifentanil.

Pain sensitivity reflects a balance between pain-facilitating and inhibitory systems and an increase in the latter component may induce a tolerance effect, whereas a hyperalgesic effect would be obtained when such increase overcomes that produced in the pain-facilitating system. With the introduction of the very short-acting remifentanil, there is evidence suggesting that tolerance rapidly develops, increasing pain perception in the postoperative period but also blunting the MAC reduction. However, a lack of development of acute opioid tolerance after remifentanil-based anaesthesia has also been reported. Glial activation, and opioid-induced increase in pro-inflammatory cytokines and chemokines, may contribute to the development of opioid tolerance. Here, we demonstrate that the studied COX inhibitors prevented opioid tolerance with paracetamol in the short term potentiating the remifentanil action on the MAC.

In conclusion, the use of COX inhibitors does not provide a consistent potentiation of the remifentanil effects on the MAC where a clear potentiation was observed with paracetamol but not with ketoprofen, metamizole, or parecoxib. However, the tolerance effect to remifentanil is blunted by these drugs, although not prevented or avoided, promoting its combined use perioperatively. These results suggest that COX inhibitors are actually a broad group including drugs with different actions and thus they would interact with remifentanil to variable degrees. Overall, the results indicate the potential of paracetamol combined with remifentanil for the perioperative use, although the demonstration of this effect in humans warrants further investigation.

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**Conflict of interest**
None declared.

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