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Aspirin Synergistically Potentiates Isoflurane Minimum Alveolar Concentration Reduction Produced by Morphine in the Rat

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Background: **The combination of opioids and nonsteroidal antiinflammatory drugs is more analgesic than the summed effect of each drug administered separately. This synergism has been used to obtain analgesia in the postoperative period at doses at which side effects are minimal. The aim of this study is to evaluate the analgesic interaction between aspirin and morphine in the rat during isoflurane anesthesia. The reduction in minimum alveolar concentration of isoflurane (MAC_{ISO}) was used as an objective measure of the analgesic potency of individual drugs and their use in combination.**

Methods: **Thirty-seven male Wistar rats were anesthetized with isoflurane in oxygen, and the MAC_{ISO} was determined before and after the intravenous administration of aspirin and morphine. Rats were administered morphine alone (1, 3, and 10 mg/kg) or morphine (1 and 3 mg/kg) and aspirin (30 mg/kg). The MAC_{ISO} was determined from alveolar gas samples at the time of tail clamp. The duration of MAC_{ISO} reduction was recorded.**

Results: **Aspirin did not have an effect on MAC_{ISO}, (average, 1.35 ± 0.1%), whereas the combination of morphine (1 and 3 mg/kg) and aspirin (30 mg/kg) produced a reduction in the dose of morphine needed to produce the same degree of MAC_{ISO} reduction. Actual $MAC_{ISO+drug}$ data were as follows: 1 mg/kg morphine, 1.17 ± 0.14%; 3 mg/kg morphine, 0.98 ± 0.15%; 1 mg/kg morphine plus aspirin, 0.90 ± 0.04%; 10 mg/kg morphine, 0.63 ± 0.13%; and 3 mg/kg morphine plus aspirin, 0.64 ± 0.06%.**

Conclusions: **The synergistic effects of aspirin and morphine allow a clinically significant reduction in the requirements of isoflurane and isoflurane plus morphine, and these drug combinations may decrease the side effects associated with the use of single higher, equianalgesic doses of these drugs. (Key words: Analgesics; animals; inhalational anesthetics.)**

OPIOIDS are routine perioperative analgesics used to reduce the dose of intravenous or inhalation anesthetic agents. Nonsteroidal antiinflammatory drugs (NSAIDs) are administered in the postoperative period and may be combined with opioids to increase analgesic potency in humans.[1–4] Clinical studies suggest a synergistic analgesic effect when opioids and NSAIDs are administered, combined with minimal side effects[5] in the postoperative period, but there is little information related to the intraoperative period.

The mechanism of action of this effect has been identified[6,7] as a synaptic interaction between opioids and NSAIDs, in which activation of the μ-receptor causes presynaptic inhibition of the γ-aminobutyric acid (GABA) transmitter release mediated by arachidonic acid metabolites.

Opioids and NSAIDs both have potential side effects. Opioids cause respiratory depression, hypotension, nausea, and vomiting,[8] whereas NSAIDs may produce hepatic and renal failure, increased bleeding, and gastrointestinal toxicity.[9] Most side effects are drug-dose related. Accordingly, a major clinical goal of the use of both groups of analgesic drugs is to maintain their analgesic effect while reducing the dose needed. To this end, combinations of individual analgesic drugs are used at drug doses less than those used when a single drug is administered.

Opioids reduce the minimum alveolar concentration (MAC) of inhalation anesthetics and this effect may be

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potentiated by combining an opioid drug with NSAIDs. For example, the potentiation of morphine visceral antinociception effect by ketorolac has been shown in laboratory studies of rats.[10] The aim of current work is to characterize the influence of aspirin on morphine reduction of isoflurane MAC (MAC_{ISO}) in rats.

Materials and Methods

After obtaining institutional animal care committee approval, the reduction of MAC_{ISO} in response to morphine and aspirin alone or combined was evaluated in rats. Isoflurane was obtained from Abbott Laboratories (Madrid, Spain), morphine sulphate was obtained from J. Navarro Laboratories (Madrid, Spain) and acetyl salicylic acid (aspirin) was obtained from Sigma Chemical Company (St. Louis, MO). Thirty-seven male Wistar rats (CRIFFA, Barcelona, Spain) weighing 331 ± 14 g were used. The unmedicated rats were placed in an induction chamber to which 5% isoflurane (Forane; Abbott Laboratories) in a continuous oxygen flow of 3 l/min was directed (Isoflurane Vaporizer Ohmeda Isotec 3; BOC Health Care, Steeton, England). Two to three minutes later, the inspired isoflurane concentration was reduced to 2.5–3%.

Tracheal intubation was performed using a 14-gauge polyethylene catheter (Abbott Ireland, Sligo, Republic of Ireland) with the animal positioned in dorsal recumbency. A cold light was applied externally over the trachea so the larynx could be observed easily *via* the oral cavity. Then a flexible, blunt-tip, wire guide was inserted into the trachea with an otoscope and used to direct the endotracheal catheter. After the correct position of the catheter was ascertained, it was connected to a small T piece of minimal dead space. Fresh gas flow to the T piece was adjusted to 1 l/min, and isoflurane concentration was adjusted as necessary by prevailing conditions.

Monitoring

The carotid artery was catheterized with a fine tubing (800/110/200; Portex, Hythe, United Kingdom) *via* surgical cut-down. This access allowed for arterial blood sampling and blood pressure measurement *via* a calibrated pressure transducer. Arterial blood pressure and electrocardiography were recorded continuously (CM-8B; Schiller AG, Baar, Switzerland). Arterial blood gases (blood gas analyzer, Statnova profile-1; Nova Biomedical, Waltham, MA) were measured, occasionally during the MAC assessment, and at the end of study period, to ensure values were within normal limits of *p*H (7.35–7.45), pressure of oxygen (P_{O_2}) (> 90 mmHg), and pressure of carbon dioxide (P_{CO_2}) (35–47 mmHg). Rectal temperature also was monitored and maintained between 37°C and 38°C by means of a circulating-water warming blanket (Heat Therapy Pump, Model TP-220; Gaymar, Orchand Park, NY) and, occasionally, a heating light.

Determination of the Minimum Alveolar Concentration

Intratracheal gas sampling was used to measure anesthetic gas concentration for determination of the MAC. This method has been described in detail previously.[11] In brief, a fine catheter (model 100/383/118; Portex, Hythe, United Kingdom) with 0.9 mm external diameter was inserted through the endotracheal catheter with the tip located at the level of the carina. The proximal end of the catheter was connected to a 10-ml gas-tight glass syringe (Gastight #1010SL; Hamilton, Reno, NV). Sampling was obtained by withdrawing 10 ml gas over 5 min using a Harvard infusion pump (Harvard Apparatus, Millis, MA). Samples were obtained consecutively in triplicate to ensure constant alveolar concentration, and the final value was the average at every isoflurane concentration step. The catheter was withdrawn between samples. After every step change in anesthetic concentration delivered by the anesthetic circuit, at least 15 min were allowed for equilibration before gas samples were obtained.[12] Gas samples were assayed using a side-stream infrared gas analyzer (5330 Agent Monitor; Ohmeda, West Yorkshire, United Kingdom).

The MAC_{ISO} value was established according to the method described by Eger *et al.*[12] A painful noxious stimulus was applied with a long hemostat (8-inch Rochester Dean Hemostatic Forceps; Martin, Tuttlingen, Germany) clamped to the first ratchet lock on the tail for 60 s while the third gas sample was obtained from the lung. The tail was always stimulated proximal to a previous test site. A positive response was considered when a gross purposeful movement of the head, extremities or body, or both, was observed, whereas a negative response was the lack of movement or grimacing, swallowing, chewing, or tail flick. The isoflurane concentration was then reduced in decrements of 0.1 to 0.15% until the negative response became positive. The MAC was considered to be the concentration midway between the highest concentration that permitted move-

Table 1. Minimum Alveolar Concentration of Isoflurane in Rats before (MAC_{ISO}) and after ($MAC_{ISO+drug}$) Receiving Morphine Alone or in Combination with Aspirin

	Morphine 1 mg/kg + Saline	Morphine 3 mg/kg + Saline	Morphine 10 mg/kg + Saline	Morphine 1 mg/kg + Aspirin	Morphine 3 mg/kg + Aspirin	Saline
MAC_{ISO}	1.40 ± 0.09	1.37 ± 0.1	1.40 ± 0.1	1.32 ± 0.06	1.29 ± 0.13	1.31 ± 0.10
$MAC_{ISO+drug}$	1.17 ± 0.14	0.98 ± 0.15	0.63 ± 0.13	0.90 ± 0.04	0.64 ± 0.06	1.31 ± 0.09
Reduction (%)	17 ± 6	29 ± 10	55 ± 8	32 ± 2	50 ± 6	−1 ± 1
n	6	6	6	6	7	6

ment in response to the stimulus and the lowest concentration that prevented movement.

Experimental Design

Basal (predrug) MAC_{ISO} was determined in every unmedicated rat. Animals then received an equal volume (1.2 ml/kg) of either saline or aspirin intravenously, MAC_{ISO} was redetermined 30 min later, and the $MAC_{ISO+aspirin}$ was determined in groups receiving aspirin. Finally rats were administered either saline (1.2 ml/kg) or morphine (see drug groups) and MAC_{ISO} was redetermined again. The total duration of the MAC_{ISO} reduction after opioid administration was defined as the time in minutes for the MAC_{ISO} to regain the basal predrug value in each rat. Minute zero of the "duration of the MAC reduction" was set to the time of intravenous morphine administration. The duration of the MAC reduction value was considered the point when a positive response to the noxious stimulus was obtained at the same maximum MAC_{ISO} as that of the MAC_{ISO} value in the unmedicated rat. When no reduction in MAC_{ISO} was observed, both times were set to 0 min. All recorded times were set to the beginning of isoflurane alveolar concentration measurement at every concentration step change since a stabilization time of 15 min was established. Then, 25–30 min was the normal time spent between two isoflurane alveolar concentration determinations.

Drug Groups

Animals receiving saline were administered one of three doses of morphine (1, 3, or 10 mg/kg) or saline (control), whereas animals receiving aspirin (30 mg/kg) were administered one of two doses of morphine (1 or 3 mg/kg). The animals were randomly, in an unblinded manner, assigned to a total of six groups ($n \geq 6$). All drugs were administered intravenously in 3–5 min to reduce cardiovascular and respiratory effects when administered more quickly. Animals receiving saline only were further tested for changes in MAC_{ISO} 60 and 120 min after the second saline administration.

Side Effects

Side effects produced by drug bolus administration on the cardiovascular (blood pressure and electrocardiography) and respiratory rate were monitored.

Statistical Analysis

Statistical analysis of data was performed using a computer software program (SPSS statistical software, Chicago, IL). Data are expressed as the mean ± SD. Analysis of variance was performed and *post hoc* comparison of the groups was performed using the Newman-Keuls test. A P value < 0.05 was set to indicate statistical significance.

Results

Minimum Alveolar Concentration of Isoflurane

The average MAC_{ISO} value determined in all the rats was 1.35% ± 0.10%. No significant differences between groups were observed. Aspirin did not modify the MAC_{ISO} value: morphine 1 mg/kg + aspirin, postaspirin MAC_{ISO} = 1.32 ± 0.05; morphine 3 mg/kg + aspirin, postaspirin MAC_{ISO} = 1.30 ± 0.13 (table 1).

Reduction of Minimum Alveolar Concentration of Isoflurane

Morphine reduced the MAC_{ISO} in a dose-dependent fashion, and the addition of aspirin produced a greater decrease in the MAC_{ISO} reduction than that observed by morphine alone. Aspirin did not produce any change in the MAC_{ISO} value in the two groups receiving aspirin before morphine administration. Group comparison showed a similar MAC_{ISO} reduction between 3 mg/kg morphine and 1 mg/kg morphine plus aspirin, (*i.e.*, a three-fold reduction in the dose of morphine needed to produce the same level of MAC_{ISO} reduction) (figure 1). Individual group MAC_{ISO} values are shown in table 1. Also pairwise comparisons between percentages of MAC_{ISO} reduction are shown in table 2.

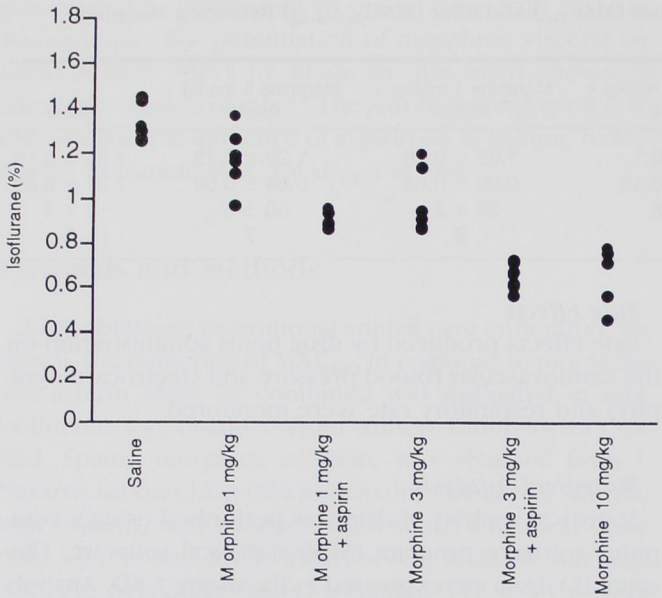

Fig. 1. Values of individual minimum alveolar concentration of isoflurane of rats administered either saline or morphine alone or in combination with aspirin.

Duration of Reduction of Minimum Alveolar Concentration of Isoflurane

The MAC_{ISO} reduction time ranged from 96 ± 19 min and 105 ± 27 min for the two lowest doses of morphine with or without aspirin administration up to 183 ± 38 min produced by 10 mg/kg morphine. Morphine, 3 mg/kg, MAC_{ISO} reduction time ranged from 112 ± 24 to 137 ± 31 min without or with aspirin, respectively.

Side Effects

Cardiovascular Effects. There were no differences in basal arterial blood pressure among the studied groups (mean arterial pressure = 101 ± 15 mmHg). A drop in arterial blood pressure was observed after the administration of morphine but not after aspirin. The magnitude of blood pressure decrease was similar in all groups receiving morphine, irrespective of the dose used. Aspirin did not modify mean arterial pressure.

Respiratory Effects. Morphine, but not aspirin, produced transient apnea (always < 5 min) and respiratory arrhythmia immediately after its administration. Values of *p*H, P_{O_2} and P_{CO_2} were within normal range throughout the study period.

Discussion

Aspirin further increased the MAC_{ISO} reduction produced by morphine administration. The observed effect is synergistic, as shown by the lack of effect of aspirin alone, although a possible intraoperative anesthetic sparing effect of NSAIDs alone has been described in humans.[13]

The comparison of the multiple doses of morphine used enables us to consider a three-fold reduction in the dose of morphine needed to produce the same level of MAC_{ISO} reduction. In a model of acute visceral pain in the rat without a major inflammatory component, it has shown a synergistic analgesic effect of ketorolac and morphine, separate from the peripheral antiinflammatory properties.[10] The morphine sparing effect was approximately 50%. A similar opioid sparing effect has been observed in postoperative patients,[2,4] and it has been shown that intraoperative ketorolac would act synergistically with fentanyl to decrease postoperative analgesic requirements in humans.[14] This study shows, for the first time, an opioid sparing effect in the perioperative period and a potential reduction in the doses of anesthetics needed. The use of the MAC value as a reference to test the analgesic potency of the opioid–NSAID combination also provides an objective assessment of their potency because most studies used a visual analog scale score or an opioid sparing effect, or both, in postoperative patients.[2,4]

The rationale for administration of opioids with NSAIDs is to diminish the dose necessary to produce a particular therapeutic endpoint and to reduce accordingly the respective side effects associated with the use of either group of agents. The analgesic effects of a combination of opioids and NSAIDs is synergistic, whereas other combinations only produce an additive analgesic effect.[15] The administration of these drugs combined is not new,[5] and they have been long recommended in cancer patients to reduce the doses of opioids necessary to achieve satisfactory pain relief.[16]

The analgesic action of NSAIDs has been explained on the basis of their inhibitory effect on the enzymes that synthesize prostaglandins. The analgesic effect is exerted through peripheral and central inhibitory effects.[17] A central mechanism of action of NSAIDs[18] has been postulated.

There are two structurally distinct forms of the cyclooxygenase enzyme (COX_1 and COX_2), where COX_2 is the inducible but also the constitutive form in many cell types, including inflammatory cells.[19] Inhibition of the COX_2 form is the more likely mechanism of action of NSAIDs, in which the relative COX_2-COX_1 inhibitory action of the different NSAIDs available determine the likelihood of side effects. The COX_2:COX_1 ratio for aspi-

Table 2. Pairwise Comparisons between Groups

	Morphine 3 mg/kg + Saline	Morphine 10 mg/kg + Saline	Morphine 1 mg/kg + Aspirin	Morphine 3 mg/kg + Aspirin	Saline
Morphine 1 mg/kg	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.05$
Morphine 3 mg/kg + saline		$P < 0.01$	NS	$P < 0.01$	$P < 0.01$
Morphine 10 mg/kg + saline			$P < 0.01$	NS	$P < 0.01$
Morphine 1 mg/kg + aspirin				$P < 0.01$	$P < 0.01$
Morphine 3 mg/kg + aspirin					$P < 0.01$

NS = not significant.

rin is considerably higher than that of newer NSAIDs. Therefore, NSAIDs with fewer side effects may be more advantageous than conventional ones, such as aspirin.

Different mechanisms of analgesic drug interaction with NSAIDs have been postulated,[15] but only recently has a possible mechanism of action of the synergistic analgesic effect of these drugs with opioids been proposed[7] based on the opioid inhibitory γ-aminobutyric acid–mediated neurotransmission in the brain.[20] Activation of the μ-receptor causes a presynaptic inhibition of γ-aminobutyric acid *via* a presynaptic voltage-dependent potassium conductance. This mechanism is mediated by a pathway involving an activation of phospholipase A_2 with production of arachidonic acid and its metabolites.[6] Nonsteroidal antiinflammatory drugs may block the prostaglandin production without affecting the production of pain-relieving lipooxygenase metabolites.[7] A significant component of MAC has now been shown to occur at the spinal cord.[21] Also, opiates and NSAIDs act at the spinal level, and evidence suggest a synergic potentiation in the response to a noxious stimulus.[15]

The dose of aspirin used in the rat may be considered in the low range. It is not known from this study whether doses smaller than 30 mg aspirin may produce a further potentiation in the reduction of the MAC value. A daily dose of 48 mg/kg in the rat is equivalent to a human daily dose of 1,300 mg and may be considered safe in the rat.[22] Analgesic (NSAID) nephropathy has been described in rats after long-term daily administration of eight times the dose used in this study (230 mg · kg^{-1} · day^{-1}).[23]

In conclusion, this study shows a perioperative anesthetic-opioid sparing effect of aspirin in the rat. This effect may reduce perioperative anesthetic and opioid drug doses. Further research is necessary to show the effect in humans, to distinguish the differences between the various NSAIDs, to show the optimal schedules and routes of administration, and, finally, to show the cost-effectiveness and influence on the quality of anesthesia.

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