Effects of Propofol and Remifentanil on Phrenic Nerve Activity and Nociceptive Cardiovascular Responses in Rabbits

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Background: The effects of propofol, remifentanil, and their combination on phrenic nerve activity (PNA), resting heart rate (HR), mean arterial pressure (MAP), and nociceptive cardiovascular responses were studied in rabbits.

Methods: Basal anesthesia and constant blood gas tensions were maintained with α-chloralose and mechanical ventilation. PNA, HR, MAP, and maximum changes in HR and MAP (ΔHR, ΔMAP) evoked by electrical nerve stimulation of tibial nerves were recorded. The comparative effects were observed for propofol at infusion rates from 0.05 to 3.2 mg·kg−1·min−1 (group I) and remifentanil from 0.0125 to 12.8 μg·kg−1·min−1 alone (group II), and during constant infusions of propofol at rates of 0.1 and 0.8 mg·kg−1·min−1 (groups III and IV, respectively). Finally, the effect of remifentanil on propofol blood levels was observed (group V).

Results: The infusion rates for 50% depression (ED50) of PNA, ΔHR, and ΔMAP were 0.41, 1.32, and 1.58 mg·kg−1·min−1 for propofol, and 0.115, 0.125, and 1.090 μg·kg−1·min−1 for remifentanil, respectively. The ratios for the ED50 values of ΔHR and ΔMAP to PNA were 3.2 and 3.9 for propofol and 1.1 and 9.5 for remifentanil, respectively. Analysis of the expected and observed responses and isobolograms showed that although their combined effects on PNA, resting HR, and MAP, and ΔMAP were synergistic for ΔHR, they were merely additive. Remifentanil had no effect on propofol blood levels.

Conclusion: PNA was abolished by propofol and remifentanil, alone and in combination, before significant depression of nociceptive pressor responses occurred. Their combined effects on PNA, HR, MAP, and ΔMAP are greater than additive, i.e., synergistic. Unlike propofol, remifentanil obsoleted pressor responses more than the resting circulation. (Key words: Anesthesia; drug interaction; respiration.)

DEPRESSION of sympathoexcitation and its consequences are important goals in anesthesia because they may improve surgical outcome.1,2 Opioids depress adrenergic responses to surgical stimuli.3,4 Remifentanil, an analog of fentanyl, is unique among opioids by virtue of its rapid clearance and brief duration of action, which, regardless of dose or the physiologic condition of the patient, is a result of the rapidity of its metabolism by blood and tissue esterases.5–8 In addition, it is unlikely to display pharmacokinetic interactions with other drugs metabolized, e.g., by hepatic enzymes. The very short constant context-sensitive half-time (approximately 3 min)9,10 provides a pharmacodynamic basis for its use in total intravenous anesthesia, e.g., in combination with propofol.11 In addition, by allowing the generation of repeated dose–response curves without cumulation, it facilitates research on the nature of the pharmacodynamic interactions of μ opioids with other drugs.

This study, conducted in a rabbit model, was designed to determine the relative effects of propofol and remifentanil on central respiratory activity assessed by phrenic nerve activity (PNA), the resting circulation, cardiovascular responses to stimulation of somatic nerves, and the nature of their pharmacodynamic interactions. Pharmacokinetic interaction was excluded by measuring blood levels of propofol during infusions of remifentanil.

Methods

General Procedure

This study was approved by the United Kingdom Home Office (PPL No. 90/00851). Experiments were performed on New Zealand White rabbits of both sexes, weighing 3.5–4.5 kg. Anesthesia was induced intravenously with 10–15 mg/kg methohexital and maintained...
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Table 1. Outline of the Experimental Groups and Examples of Data for Arterial Carbon Dioxide Partial Pressure

<table>
<thead>
<tr>
<th>Group</th>
<th>Effects of propofol alone (n = 5)</th>
<th>Infusion Rates of Propofol (mg·kg⁻¹·min⁻¹)</th>
<th>Withdrawal</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>0.05</td>
</tr>
<tr>
<td>PaCO₂</td>
<td>36 ± 2</td>
<td></td>
<td>35 ± 3</td>
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<table>
<thead>
<tr>
<th>Group</th>
<th>Effects of remifentanil alone (n = 5)</th>
<th>Infusion Rates of Remifentanil (µg·kg⁻¹·min⁻¹)</th>
<th>Withdrawal</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>0.05</td>
</tr>
<tr>
<td>PaCO₂</td>
<td>36 ± 4</td>
<td></td>
<td>35 ± 4</td>
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</tbody>
</table>

<table>
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<tr>
<th>Group</th>
<th>Combined effects of propofol (0.1 mg·kg⁻¹·min⁻¹) and remifentanil on PNA (n = 5)</th>
<th>Infusion Rates of Remifentanil (µg·kg⁻¹·min⁻¹)</th>
<th>Withdrawal</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
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</tr>
<tr>
<td>PaCO₂</td>
<td>36 ± 3</td>
<td></td>
<td>36 ± 2</td>
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<thead>
<tr>
<th>Group</th>
<th>Combined effects of propofol (0.8 mg·kg⁻¹·min⁻¹) and remifentanil on evoked cardiovascular responses (n = 5)</th>
<th>Infusion Rates of Remifentanil (µg·kg⁻¹·min⁻¹)</th>
<th>Withdrawal</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>0.05</td>
</tr>
<tr>
<td>PaCO₂</td>
<td>35 ± 3</td>
<td></td>
<td>35 ± 3</td>
</tr>
</tbody>
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<table>
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<tr>
<th>Group</th>
<th>Effect of remifentanil on blood levels of propofol (n = 5)</th>
<th>Infusion Rates of Remifentanil (µg·kg⁻¹·min⁻¹)</th>
<th>Withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>0.8</td>
</tr>
<tr>
<td>PaCO₂</td>
<td></td>
<td></td>
<td>36 ± 1</td>
</tr>
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</table>

Values are mean ± SD. Because of the critical importance in studies of central respiratory activity and the autonomic and cardiovascular systems, not only was the arterial carbon dioxide partial pressure (PaCO₂) carefully controlled but also the pHa was maintained between 7.361 and 7.410 and the PaO₂ was between 225 and 250 mmHg in all the groups. There were no significant differences in any of the blood gas data throughout these experiments.

with 1% α-chloralose (Sigma, St. Louis, MO) in an initial intravenous bolus dose of 30 mg/kg, followed by a continuous infusion of 15–20 mg·kg⁻¹·h⁻¹. The lungs were mechanically ventilated (SLE 2000, SLE Ltd, Croydon, United Kingdom) with oxygen-enriched air through a tracheal tube inserted via a tracheostomy. Muscle paralysis was maintained with bolus doses of succinylcholine (Wellcome Foundation Ltd., London, United Kingdom) (2 mg/kg intravenously every 20–30 min). A femoral artery was cannulated for recording arterial pressure and sampling blood, and a femoral vein was also cannulated for the infusion of 1% α-chloralose and 0.9% saline. A catheter was inserted near the right atrium via the right external jugular vein for measuring central venous pressure. The marginal veins of both ears were cannulated for the infusion of drugs. Esophageal temperature was measured with a thermistor (Yellow Springs Instruments, Yellow Springs, OH) and maintained between 37°C and 38°C using a heating system in the operating table. Arterial pH (pHa) and arterial blood gas tensions were measured using a blood gas analyzer (Radiometer ABL 3, Copenhagen, Denmark). They were maintained throughout the study at pHa 7.36–7.4, arterial carbon dioxide tension 35–37 mmHg, and arterial oxygen tension 225–250 mmHg (table 1) by adjusting the tidal volume, without changing the frequency of ventilation and occasionally by the administration of small doses of sodium bicarbonate. The resting mean arterial pressure (MAP), heart rate (HR), and evoked changes in HR (ΔHR) and MAP (ΔMAP) were recorded on a multichannel recording system and stored on a disk for subsequent analysis (Maclab 8, ADInstruments, Castle Hill, Australia).

PNA
The right phrenic nerve was exposed in the neck by a ventral approach; a portion was dissected from adjacent tissues, a short section of which was desheathed and cut

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distally, immersed in a pool of mineral oil, and mounted on silver electrodes to record efferent activity. Signals from the phrenic nerve were preamplified (Tektronix 122, Beaverton, OR), and displayed on a dual-beam oscilloscope (Tektronix 565). The amplified signals were rectified and integrated with a 100-ms time constant (Neurolog N190, Hertfordshire, United Kingdom). Both amplified and integrated signals were also displayed on an oscilloscope and plotted with a thermal recorder (Gould 1602, Essex, United Kingdom). The total electrical activity of the rectified and integrated signals during 20 s periods was measured in arbitrary units. In vagally intact animals, PNA will synchronize with the ventilator at normal respiratory rates. However, in some animals receiving higher dose of remifentanil, bursts of PNA fell below the ventilator rate before total abolition of activity. To eliminate the distortion of the PNA signals by the artefact from trains of electrical stimuli applied to the tibial nerve at each stage of the study and at 5, 20, and 30 min after the start of the propofol infusion, when its effect was stable, an infusion of remifentanil was started at increasing infusion rates using the same protocol as in group 11. In group IV (n = 5), propofol was administered intravenously with an initial bolus dose of 2 mg/kg, followed by a continuous infusion at a rate of 0.1 mg·kg⁻¹·min⁻¹, each for 15 min until the effect of the drug at each infusion rate was stable, because previous studies have shown that an infusion of remifentanil at a constant rate produced a constant blood level within 15 min. The infusion rates of both drugs were chosen after pilot studies that showed that the evoked cardiovascular responses would be almost abolished at the highest infusion rates. In group III (n = 5), propofol was administered intravenously with an initial bolus dose of 0.5 µg/kg, followed by a continuous infusion at a rate of 0.0125 to 12.8 µg·kg⁻¹·min⁻¹, each for 15 min until the effect of the drug at each infusion rate was stable, because in group I, it depressed PNA by approximately 20%, thereby allowing the effect of the addition of remifentanil to be observed. Fifteen minutes after the start of the propofol infusion, when its effect was stable, an infusion of remifentanil was started at increasing infusion rates using the same protocol as in group II. In group I (n = 5), propofol was administered intravenously with an initial bolus dose of 2 mg/kg, followed by a continuous infusion at a rate of 0.8 mg·kg⁻¹·min⁻¹ that abolished PNA. This rate of infusion was selected because in group I, it depressed PNA by approximately 25%, thereby allowing the effect of the addition of remifentanil on ΔHR and ΔMAP to be observed at increasing infusion rates using the group II protocol. Measurements were repeated at the end of each stage of the study and at 5, 20, and 30 min after simultaneous termination of the administration of both drugs. Groups III and IV demonstrated the nature of the interaction between propofol and remifentanil, i.e., whether they are additive, synergistic, or antagonistic in their effects on PNA, evoked cardiovascular responses, and the resting circulation. Their interactions were examined by the methods summarized by Berenbaum, involving measurement of the effect of a fixed dose of one drug on the dose–response curve of the other and also the construction of isobolograms. Finally, in group V (n = 5), propofol was administered intravenously with an initial bolus of 2 mg/kg, followed by a continuous infusion for 105 min at a rate of 0.8 mg·kg⁻¹·min⁻¹, which reduced the mean pressor responses to tibial

Evoked Changes in HR and MAP

The tibial nerve in the right hindleg was exposed in the upper to middle part of the thigh. A portion was dissected free from the surrounding tissues, and a short length (approximately 1.5 cm) was desheathed, cut distally, and mounted on silver electrodes in a mineral oil pool for electrical stimulation. Five-second trains of supramaximal electrical stimuli (30 Hz; 30 V; stimulus duration, 0.5 ms) were applied to the tibial nerve (S88 stimulator; Grass, Quincy, MA). The stimulus frequency, intensity, and duration were determined from a pilot study that demonstrated that the stimulus intensity was supramaximal for the evoked cardiovascular responses and that a train duration of 5 s was sufficient to evoke maximal responses but not sufficiently long for the baroreflexes, responding to the increase in blood pressure, to cause a reduction in HR from its maximum value. The maximum increases in HR (ΔHR) and MAP (ΔMAP) in response to stimulation were recorded.

Experimental Design

Twenty-five rabbits were assigned randomly to one of five equal groups as summarized in table 1. Each animal was allowed to stabilize for at least 30 min after completion of surgery before starting the study, after which control measurements were obtained and repeated 20-30 min later to ensure that the control baseline data had not changed. Animals in group I (n = 5) were given propofol (Zeneca Ltd., Cheshire, United Kingdom) in an initial intravenous bolus dose of 2 mg/kg, followed by a continuous infusion at incrementally increasing rates from 0.05 to 3.2 mg·kg⁻¹·min⁻¹. Each infusion rate was maintained for 15 min to ensure stable recordings. In group II (n = 5), remifentanil (Glaxo Wellcome, Middlesex, United Kingdom) was administered intravenously with an initial bolus dose of 0.5 µg/kg, followed by continuous infusion rates from 0.0125 to 12.8 µg·kg⁻¹·min⁻¹, each for 15 min until the effect of the drug at each infusion rate was stable, because previous studies have shown that an infusion of remifentanil at a constant rate produced a constant blood level within 15 min. The infusion rates of both drugs were chosen after pilot studies that showed that the evoked cardiovascular responses would be almost abolished at the highest infusion rates. In group III (n = 5), propofol was administered intravenously with an initial bolus dose of 0.5 µg/kg, followed by a continuous infusion at a rate of 0.1 mg·kg⁻¹·min⁻¹, each for 15 min until the effect of the drug at each infusion rate was stable, because in group I, it depressed PNA by approximately 20%, thereby allowing the effect of the addition of remifentanil to be observed. Fifteen minutes after the start of the propofol infusion, when its effect was stable, an infusion of remifentanil was started at increasing infusion rates using the same protocol as in group II. In group IV (n = 5), propofol was administered intravenously with an initial bolus dose of 2 mg/kg, followed by a continuous infusion at a rate of 0.8 mg·kg⁻¹·min⁻¹ that abolished PNA. This rate of infusion was selected because in group I, it depressed PNA by approximately 25%, thereby allowing the effect of the addition of remifentanil on ΔHR and ΔMAP to be observed at increasing infusion rates using the group II protocol. Measurements were repeated at the end of each stage of the study and at 5, 20, and 30 min after simultaneous termination of the administration of both drugs. Groups III and IV demonstrated the nature of the interaction between propofol and remifentanil, i.e., whether they are additive, synergistic, or antagonistic in their effects on PNA, evoked cardiovascular responses, and the resting circulation. Their interactions were examined by the methods summarized by Berenbaum, involving measurement of the effect of a fixed dose of one drug on the dose–response curve of the other and also the construction of isobolograms. Finally, in group V (n = 5), propofol was administered intravenously with an initial bolus of 2 mg/kg, followed by a continuous infusion for 105 min at a rate of 0.8 mg·kg⁻¹·min⁻¹, which reduced the mean pressor responses to tibial
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Table 2. ED₅₀ (95% Confidence Intervals) Values for Infusion of Propofol (mg·kg⁻¹·min⁻¹) and Remifentanil (µg·kg⁻¹·min⁻¹), Alone and in Combination, on Phrenic Nerve Activity (PNA), and Changes in HR and MAP (ΔHR and ΔMAP) Evoked by 5-s Trains of Supramaximal High Frequency Electrical Stimulation (30 Hz, 30V, duration 0.5 ms) of Tibial Nerves, and the Ratios of the ED₅₀S for ΔHR and ΔMAP to PNA

<table>
<thead>
<tr>
<th></th>
<th>Propofol Alone ED₅₀</th>
<th>Remifentanil Alone ED₅₀</th>
<th>Predicted ED₅₀ for Remifentanil When Combined with Propofol</th>
<th>Observed ED₅₀</th>
<th>P/O</th>
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<tbody>
<tr>
<td>PNA</td>
<td>0.41 (0.32–0.65)</td>
<td>0.115 (0.088–0.142)</td>
<td>0.087</td>
<td>0.050 (0.040–0.058)</td>
<td>1.7/1</td>
</tr>
<tr>
<td>ΔHR</td>
<td>1.32 (0.98–2.03)</td>
<td>0.125 (0.080–0.156)</td>
<td>0.052</td>
<td>0.050 (0.029–0.081)</td>
<td>1/1</td>
</tr>
<tr>
<td>ΔMAP</td>
<td>1.58 (1.21–2.35)</td>
<td>1.090 (0.810–1.520)</td>
<td>0.500</td>
<td>0.138 (0.100–0.224)</td>
<td>3.6/1</td>
</tr>
</tbody>
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<table>
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<tr>
<th></th>
<th>Ratios of ED₅₀</th>
<th>Ratios of ED₅₀</th>
<th>Ratios of ED₅₀</th>
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<tbody>
<tr>
<td>ΔHR/PNA</td>
<td>3.2/1</td>
<td>1.1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>ΔMAP/PNA</td>
<td>3.9/1</td>
<td>9.5/1</td>
<td>2.8/1</td>
</tr>
</tbody>
</table>

The predicted (P) and observed (O) ED₅₀S refer to remifentanil when combined with infusions of propofol at rates of 0.1 mg·kg⁻¹·min⁻¹ for PNA and 0.8 mg·kg⁻¹·min⁻¹ for ΔHR and ΔMAP, and their ratio (P/O).

nerve stimulation by approximately 25%. Forty-five minutes after starting the propofol infusion, remifentanil was introduced at an infusion rate of 0.8 µg·kg⁻¹·min⁻¹, which was subsequently increased to 1.6 and 3.2 µg·kg⁻¹·min⁻¹, each for 15 min, after which it was withdrawn, i.e., 15 min before the withdrawal of propofol. Control blood samples (2 ml) were obtained before starting the propofol infusion; during the infusion at 10, 15, 30, 45, 60, 75, 90, and 105 min; and 30 min after propofol withdrawal. The loss of blood caused by sampling was replaced by 0.9% saline (20–23 ml). Samples were kept in EDTA tubes and stored at 4°C.

Propofol Analysis

The concentration of propofol in whole blood was analyzed by a modified solvent extraction technique with an internal standard, quality control, and subsequent quantification and measured by gas liquid chromatography (Hewlett-Packard HP5980 fitted with a flame ionization detector, autosampler, and DB1701 megabore column; Palo Alto, CA). The lower limit of detection was 0.05 µg/ml, and the coefficient of variation was 3.0%. As yet there is no standard reference for this technique.

Data Analysis

Changes in the average of three measurements of PNA, i.e., 3 × 20-s periods measured in arbitrary units, and three groups of evoked cardiovascular responses, i.e., ΔHR and ΔMAP, were expressed as a percentage of control. Data are presented as mean ± SD and ED₅₀ (95% confidence limits). Statistical analysis was performed by analysis of variance followed, where indicated, by paired t tests with Bonferroni correction. P < 0.05 was considered to be statistically significant. Regression analysis of logarithmic plots was used to determine the slope and 95% confidence limits of the dose-response curves. The ED₅₀ values for depressant effects on PNA, ΔHR, and ΔMAP were calculated from the dose-response curves together with 95% confidence limits. The predicted effects of ED₅₀ values for remifentanil during a constant infusion of propofol 0.1 mg·kg⁻¹·min⁻¹ for PNA and 0.8 mg·kg⁻¹·min⁻¹ for cardiovascular responses (table 2), assuming their effects were additive, were calculated according to the following equation:

\[ \frac{a}{(ED_{50})} x + \frac{b}{(ED_{50})} y = 1 \]

where a and b are the doses for drug x and y during concurrent administration. Isobolograms, prepared by plotting the ED₅₀ values of both drugs, were used to show the nature of their combined effects. The additive data for MAP and HR were the sum of the mean effects of propofol and remifentanil at infusion rates of 0.8 mg·kg⁻¹·min⁻¹ and 3.2 µg·kg⁻¹·min⁻¹, respectively (table 3).

Results

Examples of recordings showing the effects of propofol on PNA and evoked circulatory responses to tibial nerve stimulation are shown in figure 1.

Resting HR and MAP

The effects of propofol on HR became significant at 3.2 mg·kg⁻¹·min⁻¹ with a decrease from the mean control value of 245 ± 31 beats/min to 228 ± 35 beats/min (P <
Table 3. Reduction in Resting Heart Rate (beats/min) and Mean Arterial Pressure (mmHg) Caused by Propofol and Remifentanil, Alone and in Combination

<table>
<thead>
<tr>
<th></th>
<th>Propofol (0.8 mg · kg⁻¹ · min⁻¹)</th>
<th>Remifentanil (3.2 µg · kg⁻¹ · min⁻¹)</th>
<th>Propofol + Remifentanil (0.8 mg · kg⁻¹ · min⁻¹) + (3.2 µg · kg⁻¹ · min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease in HR (%)</td>
<td>8 ± 4</td>
<td>13 ± 4</td>
<td>Predicted Observed</td>
</tr>
<tr>
<td>Decrease in MAP (%)</td>
<td>5 ± 2</td>
<td>21 ± 6</td>
<td>26 ± 5</td>
</tr>
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</table>

Values are mean ± SD (n = 5). Comparison of their predicted combined effects, if these were additive: *P < 0.05 and †P < 0.01. Their combined effects were synergistic.

HR = heart rate; MAP = mean arterial pressure.

0.05). Mean MAP decreased from 90 ± 13 mmHg to 46 ± 13 mmHg (P < 0.01) by 3.2 mg · kg⁻¹ · min⁻¹ (fig. 2A). For remifentanil, the maximum reductions in mean HR and MAP from control values of 260 ± 28 beats/min and 89 ± 11 mmHg to 202 ± 18 beats/min (P < 0.05) and 67 ± 5 mmHg (P < 0.01), respectively, occurred at the highest infusion rate (12.8 mg · kg⁻¹ · min⁻¹) (fig. 2B).

During combined administration, two infusion rates of propofol were used. At the lower rate (0.1 mg · kg⁻¹ · min⁻¹), remifentanil up to a rate of 0.4 µg · kg⁻¹ · min⁻¹ did not affect HR and MAP (fig. 2C); at the higher rate (0.8 mg · kg⁻¹ · min⁻¹), remifentanil caused significant and progressive decreases in both mean HR and MAP (fig. 2D).

PNA

Propofol and remifentanil caused an infusion rate-dependent depression of mean PNA that was abolished at 3.2 mg · kg⁻¹ · min⁻¹ for propofol and 0.8 µg · kg⁻¹ · min⁻¹ for remifentanil (figs. 3A and 3B). During a 15-min infusion of propofol at 0.1 mg · kg⁻¹ · min⁻¹, when the mean PNA was depressed to approximately 80% of control, the dose of

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Fig. 1. (A) Effect of propofol on PNA in one preparation. Upper traces for each pair show rectified integral of the recorded activity; the lower ones depict directly recorded PNA. (B) Effects of propofol in the same preparation on the resting circulation and evoked cardiovascular responses. The upper traces of each pair show evoked changes in heart rate (HR); the lower ones show evoked changes in arterial pressure (AP). Start of 5-s trains of electrical stimuli (30 Hz; 30 V; duration, 0.5 ms) applied to a tibial nerve is indicated by the arrows.
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**Fig. 2.** Effects of propofol (A) and remifentanil (B) and their combination (C and D) on resting mean heart rate (HR, circles; beats/min) and mean arterial pressure (MAP, squares; mmHg). Mean (SD, n = 5). Comparison with control: *P* < 0.05; **P** < 0.01.

Remifentanil required to abolish PNA was reduced by 50% to 0.4 μg·kg⁻¹·min⁻¹ (fig. 3B).

**Evoked Changes in HR and MAP**

Propofol caused dose-related depression of mean values for ΔHR and mean ΔMAP to 40% and 20% of control, respectively, at 3.2 mg · kg⁻¹ · min⁻¹ (figs. 4A and 4B). Remifentanil alone depressed ΔHR and ΔMAP to 22% and 20% of control values, respectively, at the highest infusion rate of 12.8 μg · kg⁻¹ · min⁻¹ (figs. 4C and 4D). In contrast, during propofol infusion at 0.8 mg · kg⁻¹ · min⁻¹, ΔHR was reduced to 20% of control, and ΔMAP

**Fig. 3.** Effects of (A) propofol (circles) and (B) remifentanil alone (squares) and in combination with propofol 0.1 mg · kg⁻¹ · min⁻¹ (triangles) on mean phrenic nerve activity (PNA). Mean (SD, n = 5). Comparison with control: *P* < 0.05; **P** < 0.01; ***P** < 0.001.
was almost abolished by remifentanil at 3.2 μg·kg⁻¹·min⁻¹ (figs. 4C and 4D).

**Analysis of the Nature of the Interaction Between Propofol and Remifentanil**

The expected and observed effects of propofol and remifentanil alone and in combination on PNA, ΔMAP (table 2 and fig. 5), resting HR, and MAP (table 3) were synergistic, and for ΔHR they were additive (table 2).

**Blood Concentrations of Propofol**

The mean concentration of propofol increased from 0 to 12.6 ± 2.7 μg/ml and 17.1 ± 2.6 μg/ml at 10 min and 15 min after starting its infusion, respectively, and there-

remifentanil in combination with propofol. The observed mean ED₅₀ values for PNA (A) and ΔMAP (C) when remifentanil is combined with propofol are below and to the left of the additive line, whereas for mean ΔHR (B), it is on this line, indicating that their interactions on PNA and ΔMAP are synergistic, whereas for ΔHR they are additive.

Fig. 5. Isobolograms for the combination of propofol and remifentanil on (A) mean phrenic nerve activity (PNA) and reflexly evoked changes in (B) mean heart rate (ΔHR) and (C) mean arterial pressure (ΔMAP). The solid diagonal lines are the additive lines constructed by joining the ED₅₀ values (95% confidence intervals indicated on the axes) for each drug. The points on the lines are the predicted ED₅₀, infusion rates of remifentanil causing depression of mean PNA (open triangles), ΔHR (open squares), and ΔMAP (open circles) if its interactions with propofol are additive. The symbols (filled triangles, PNA; filled squares, mean ΔHR; filled circles, ΔMAP) are the observed ED₅₀ values (95% confidence intervals indicated) for the effects of remifentanil in combination with propofol.

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interactions between propofol and remifentanil such that the drug combination abolished both PNA and ΔMAP. Thus, there was no evidence of a pharmacokinetic contribution to their interactions.

Phrenic nerve activity is a good indicator of the activity of the respiratory control system and has been used previously to observe the effect of drugs on respiration. Central and peripheral chemoreceptors have a major role in the normal control of ventilation. Blood gas tensions, pH, and temperature were carefully controlled to eliminate contributions by random physiologic variables to the changes in PNA. In addition, a return to control values after withdrawal of the test drugs was mandatory.

Discussion

This study, conducted in anesthetized, paralyzed, and artificially ventilated rabbits, shows that PNA is three to four times more sensitive to the depressive effect of propofol than either the HR or blood pressure changes evoked by supramaximal electrical stimulation of a tibial nerve (ΔHR and ΔMAP). In contrast, the effect of remifentanil on PNA and ΔHR were each about nine times greater than that for ΔMAP, indicating the much greater relative sensitivity of central respiratory activity and HR to the opioid. Although their combined effects on PNA, ΔMAP, resting HR, and MAP were synergistic, they were merely additive for ΔHR. The mean blood level of propofol at a constant infusion rate of 0.8 mg·kg⁻¹·min⁻¹, which caused major reductions in PNA and MAP, was not changed by the introduction of concurrent pharmacodynamically effective infusions of remifentanil.
diovascular responses, spontaneous ventilation is not a viable option.

Effects on the Resting Circulation

The decrease in MAP caused by propofol is due to a decrease in both cardiac output and systemic vascular resistance, and the latter is thought to be primarily a result of its depressive effect on sympathetic vasoconstrictor activity. In keeping with previous work, the present study also showed that remifentanil, at the highest doses, caused a reduction in HR and MAP and that the combined effects of remifentanil and propofol on resting HR and MAP are synergistic, which is also true for the combined effects of propofol and alfentanil in humans.

The effect of the fentanyl group of opioids on the circulation is caused by baroreflex sensitization and central effects on the autonomic nervous system, both sympathetic and parasympathetic, with depression of both MAP and HR. A ceiling effect occurs at doses that just abolish C fiber-mediated somatosympathetic reflexes, which is the same for fentanyl, alfentanil, and sufentanil in doses of approximately equal potency. Pilot studies showed that the ceiling effect of remifentanil on the resting circulation occurred at 12.8 µg · kg⁻¹ · min⁻¹ because there was no further effect beyond this, e.g., up to 25.6 µg · kg⁻¹ · min⁻¹.

Propofol, Opioids, and Synergy

To our knowledge, there is as yet no report of a pharmacokinetic interaction between propofol and remifentanil, which was confirmed in the present study and is predictable because they are metabolized in different locations by different enzymes. However, there are metabolic interactions between propofol and other opioids that affect their kinetics. For example, during combined infusions of propofol and alfentanil in man, the mean propofol blood concentration is 22% greater than that for propofol alone, and the alfentanil plasma concentrations are higher than those during the administration of alfentanil alone. This is a result of interaction at hepatic microsomal cytochrome P450 enzymes.

In addition, 50% elevation of blood propofol concentrations has been reported in patients who received an intravenous bolus dose of fentanyl (100 µg), a result of reduced uptake of propofol in the lungs caused by fentanyl. In humans, there is no evidence of pulmonary accumulation of remifentanil, and coadministration of esmolol has no significant effect on its pharmacokinetics in rats, even though both drugs are metabolized by the same nonspecific esterases. Nociceptive pressor responses are mediated by the sympathetic nervous system and, together with depression of PNA, indicate that the interactions between propofol and remifentanil occur largely in the central nervous system.

Limitations

First, a rabbit model was used in the present study. All recently developed drugs, whatever their origin, have progressed via studies on animals to clinical trials. Hence, the effects of propofol and remifentanil on the rabbit’s central nervous system are likely to have more in common with humans than otherwise. Second, there are those who consider that the depressant effect of anesthetics on nociceptive pressor responses is mediated by hypotension and peripheral vasodilatation. However, there is also a major increase in sympathetic activity when a decrease in blood pressure is induced by sodium nitroprusside. Thus, although a greater response in MAP is available, the higher level of spontaneous sympathetic activity may cause a change in the somatosympathetic reflex so that there is no net increase in MAP. For opioids such as fentanyl, a major contribution to depression of nociceptive reflexes is caused by actions on the afferent pathway in the dorsal horn region, and when administered intrathecally, they are without measurable effect on efferent sympathetic activity. Third, some critics suggest that the use of α-chloralose as a basal anesthetic compromises the results. However, it provides adequate anesthesia while preserving neural and cardiovascular reflexes with only minimal changes in the resting circulation and has no significant effect on PNA. Fourth, a valid criticism could be that an increase in MAP, acting through the baroreflexes, causes a marked depression of PNA and \textit{vice versa}. Therefore, in this study, the observations on PNA may have underestimated central respiratory depression during periods of hypotension, but this would not affect the synergistic nature of the drug interactions. Finally, it has been reported that the peak height, rather than integrated PNA signals, is a better index of muscle activity and muscle force output in spontaneously breathing animals. However, in paralyzed and artificially ventilated animals, muscle force output is not a parameter that can be considered as an index of respiratory activity. Therefore, the measurement of PNA, which is the main index of central respiratory activity in this type of study, has been reported here. Moreover, the PNA signal occurs in expiration and is terminated during inflation of lungs, at least in part by the Hering-Breuer reflex, and restarts during the expiratory pause. The thresholds for both events and the
size of the integrated signal will be changed by anesthetic drugs. At similar blood gas tensions, an increase in respiratory rate per se causes an increase in the frequency of the bursts of PNA with a consequent reduction in the signal height, which is also depressed by positive end-expiratory pressure, whereas changes in I:E ratios can affect their shape.45

In conclusion, both drugs cause much greater depression of PNA than pressor responses. For the same depression of pressor responses, remifentanil caused much less depression of the resting circulation and much greater depression of PNA than did propofol. However, it interacts synergistically with propofol to facilitate their antinociceptive effects. The results would suggest that when these drugs are used in combination for total intravenous anesthesia, a major reduction of nociceptive cardiovascular reflexes will be associated with severe respiratory depression. However, providing hypnosis is maintained, this study would indicate that a dosing strategy using proportionately more remifentanil than propofol to facilitate the abolition of adverse cardiovascular responses will cause less depression of the resting circulation.

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