PROPOFOL POTENTIATES BOTH PRE- AND POSTSYNAPTIC EFFECTS OF VECURONIUM IN THE RAT HEMIDIAPHRAGM

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SUMMARY

We have measured twitch tension in response to train-of-four stimulation in rat isolated phrenic nerve-hemidiaphragm preparations. Propofol inhibited nerve evoked twitch tension, with 50% inhibition occurring at 420 (SD 29) μmol litre⁻¹. Although propofol 100 μmol litre⁻¹ by itself had no effect on nerve evoked twitch tension, it potentiated the neuromuscular blocking effects of vecuronium. The decrease in train-of-four ratio with vecuronium was directly proportional to the degree of twitch inhibition, regardless of whether twitch was depressed by vecuronium alone or in combination with propofol. The finding that the train-of-four ratio was a function of the degree of block, rather than simply a function of vecuronium concentration, indicates that propofol also contributed to train-of-four fade and potentiated both pre- and postsynaptic effects of the neuromuscular blocker. The concentrations of propofol used in this study are much greater than human therapeutic blood concentrations, which are typically 25–35 μmol litre⁻¹ (4–6 μg ml⁻¹) immediately after a bolus dose of 2 mg kg⁻¹, suggesting that neither muscle weakness nor potentiation of vecuronium-induced neuromuscular block should be of concern at propofol concentrations occurring clinically.

KEY WORDS

Propofol has a low aqueous solubility and should be diluted in a carrier vehicle for i.v. administration. It was formulated initially using Cremophor EL, but this was not popular because of concern over potential anaphylactoid reactions [1]. Propofol is now marketed as an emulsion (Diprivan) with 10% Intralipid.

Clinical studies of propofol interaction with neuromuscular blocking agents have been complicated by effects of the vehicle. Earlier studies initially showed that propofol in Cremophor potentiated neuromuscular block produced by atracurium and vecuronium [2]. In vitro experiments confirmed that propofol in Cremophor potentiated non-depolarizing neuromuscular blocking agents [3] and decreased the amplitude of acetylcholine-induced muscle contractures [4].

Apparent interactions between propofol and neuromuscular blocking agents may have been caused by the Cremophor vehicle rather than the propofol. Clinical studies using propofol emulsion (Diprivan) failed to demonstrate any significant effects of propofol on neuromuscular block produced by suxamethonium, atracurium or vecuronium [5–6]. Fragen and co-workers [3] found that the Cremophor vehicle antagonized vecuronium and pancuronium in vitro and suggested that Cremophor inhibited acetylcholinesterase. Additional experiments with propofol in Cremophor demonstrated an increase in amplitude of nerve-evoked twitches [4] consistent with cholinesterase inhibition. Gramstad, Lilleaasen and Minsaas [7] found that Cremophor reduced the time to onset of pancuronium block and suggested that the mechanism may involve displacement of pancuronium from its plasma protein binding sites.

The present experiments were undertaken to determine the effect of propofol alone, without vehicle, on nerve evoked twitch tension and to assess the effects of propofol on vecuronium-induced neuromuscular block. Experiments were performed on the rat isolated phrenic nerve-hemidiaphragm preparation, which provides an in vitro model for studies on train-of-four fade produced by non-depolarizing neuromuscular blocking drugs [8].

MATERIALS AND METHODS

These studies were approved by the Animal Care and Use Committees at both the University of Iowa and the VA Medical Center. An isolated rat phrenic nerve-hemidiaphragm preparation modified after Bübring [9] was used. Hemidiaphragms with 2 cm of attached phrenic nerve were removed from pentobarbitone-anesthetized Sprague–Dawley rats of 200–300 g body weight. The costal margin of the hemidiaphragm was clamped in a Harvard phrenic nerve electrode and the tendinous portion was sutured to a force transducer with 00 silk. The attached nerve was clamped to stimulating electrodes. The entire assembly was placed in a 35 °C temperature-controlled organ bath and superfused with buffered mammalian Krebs solution consisting of sodium chloride 115 mmol litre⁻¹, potassium chloride 5 mmol litre⁻¹, calcium chloride

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PROPOFOL AND TWITCH TENSION

2 mmol litre$^{-1}$, magnesium sulphate 2 mmol litre$^{-1}$, sodium bicarbonate 22 mmol litre$^{-1}$, sodium dihydrogen phosphate 1.7 mmol litre$^{-1}$, HEPES buffer (4-(2-hydroxyethyl)-1-piperazineethane-sulphonic acid) 5 mmol litre$^{-1}$, glucose 11 mmol litre$^{-1}$, regular insulin 5 u. litre$^{-1}$, bubbled with 5% carbon dioxide in oxygen at pH 7.45. The preparation was rinsed initially several times with Krebs solution, to ensure that pentobarbitone was washed from the tissues.

The phrenic nerve was stimulated supramaximally by a Grass S48 stimulator in a train-of-four fashion (four stimuli each of 0.15 ms duration at 2 Hz followed by a 10-s interval). Isometric twitch tension was measured using a Grass FT 03 force displacement transducer and recorded on a Beckman R511 Dynograph. Twitch tension remained stable for 20 min before any study commenced.

A stable aqueous stock solution of propofol approximately 500 μmol litre$^{-1}$ was prepared by sonicating propofol (2,6-diisopropylphenol 97%, Aldrich) 2 μl in warmed HEPES-buffered Krebs solution 21.0 ml. The propofol was considered to be fully dissolved when the solution was clear and could transmit light without scattering caused by undissolved particles in suspension (Tyndall effect). This mixture was assayed by gas chromatography to confirm that the actual propofol concentration of the stock solution was 493 μmol litre$^{-1}$.

Dose-response (concentration-response) curves for propofol were constructed by serial addition to the bath of small aliquots of propofol or propofol stock. Every 20 min, the concentration of propofol was increased by 100 μmol litre$^{-1}$. After exposure to the greatest concentration of drug, the diaphragm was rinsed with Krebs solution to verify that twitch tension returned to within 5% of initial values.

Dose-response curves for vecuronium were obtained by increasing the concentration of vecuronium (from 0 to 6.27 μmol litre$^{-1}$) in the muscle bath every 15 min. No more than eight concentrations were applied to any single diaphragm to minimize time-dependent deterioration of the preparation. After the greatest and final concentration, the muscle bath was rinsed with fresh Krebs solution until the twitch tension returned to within 5% of initial values and twitch four (T4) was equal to twitch one (T1). Propofol was then added to the bathing solution to produce a concentration of 100 μmol litre$^{-1}$. The concentration of vecuronium was increased every 15 min to determine the vecuronium dose-response in the presence of propofol.

Cumulative dose-response (concentration-response) curves for propofol alone, vecuronium alone and vecuronium in the presence of propofol 100 μmol litre$^{-1}$ were constructed and fitted by linear regression analysis. The dose-response curves were used to determine ED50 values, where the ED50 was the concentration that inhibited maximum twitch height by 50%. Comparison of the ED50 values and the slopes of the dose-response curves for vecuronium alone and in the presence of propofol were performed using analysis of variance and Student’s t test (for unpaired or paired data as required). $P < 0.05$ was considered significant.

RESULTS

The initial set of experiments involved exposure of the hemidiaphragm preparation to increasing concentrations of propofol to determine if propofol alone, without Cremophor or lipid emulsion, affected nerve evoked twitch tension or train-of-four. Propofol inhibited twitch tension in a concentration dependent manner (fig. 1). T1 and T4 were decreased to the same degree. The propofol concentration that inhibited twitch tension by 50% was 429 (SD 29) μmol litre$^{-1}$ for T1 and 410 (29) μmol litre$^{-1}$ for T4 (n = 6 diaphragms) ($P > 0.24$, paired Student’s t test). T1 and T4 were identical at all concentrations of propofol; propofol did not produce train-of-four fade.

Another series of experiments was designed to determine if a relatively small concentration of propofol, one which had no effect by itself, would alter maximal twitch tension in response to vecuronium. A propofol concentration of 100 μmol litre$^{-1}$ was chosen because it was the maximum concentration that showed no effect on twitch tension in previous experiments. The nerve diaphragm preparation was exposed initially to increasing concentrations of vecuronium to generate a dose-response curve. The vecuronium was then washed off. When twitch had returned to control, propofol was added to the organ bath to produce a concentration of 100 μmol litre$^{-1}$. The vecuronium dose-response curve was then repeated. Bath propofol shifted the vecuronium dose-response curve for both T1 and T4 to the left (fig. 2).

The vecuronium concentration required to inhibit T1 by 50% (ED50) was reduced from 4.69 (0.36) μmol litre$^{-1}$ with vecuronium alone to 3.43 (0.41) μmol litre$^{-1}$ in the presence of propofol 100 μmol litre$^{-1}$. The vecuronium concentration required to inhibit T4 by 50% was reduced from 4.07 (0.29) μmol litre$^{-1}$ with vecuronium alone to 2.75 (0.28) μmol litre$^{-1}$ in the presence of propofol 100 μmol litre$^{-1}$ (n = 7). In each case the reduction in ED50 was statistically significant ($P < 0.01$, paired Student’s t test).

![Fig. 1. Relationship between twitch inhibition and propofol concentration for hemidiaphragms exposed to increasing concentrations of propofol. The phrenic nerve was stimulated by train-of-four and twitch tension was measured with a force transducer. Each point is the mean of n = 6 diaphragms; error bars represent SD. T1 (——) and T4 (——) are the first and fourth twitches of a train-of-four.](http://bja.oxfordjournals.org)
It is conceivable that the apparent enhancement of twitch inhibition observed with propofol may have been caused by the presence of residual vecuronium that was not washed out completely after the first set of dose–response curves had been derived. To test for this possibility, a second set of vecuronium dose–response curves was generated after washout, but in the absence of added propofol (fig. 3). The second set of vecuronium dose–response curves superimposed closely on the first set for both T1 and T4, indicating that residual vecuronium was not responsible for the effects attributed to propofol.

Propofol potentiation of vecuronium-induced depression of twitch tension was different for T1 and T4. In the absence of propofol, vecuronium decreased the train-of-four ratio (T4:T1), as expected of a non-depolarizing neuromuscular blocker. With propofol 100 μmol litre⁻¹ in the bath, T4:T1 was decreased even further, in parallel with the decrease in twitch tension (fig. 4). Propofol must have contributed to the train-of-four fade by depressing T4 to a greater extent than T1.

Depression of the train-of-four ratio was dependent solely on the total extent of twitch depression. Propofol did not affect either the slope or the position of the regression line for T4:T1 as a function of the degree of overall twitch inhibition: for a given degree of twitch inhibition, train-of-four ratio was identical regardless of whether or not twitch was inhibited by vecuronium alone or the combination of vecuronium with propofol (fig. 5).

Propofol produced a further decrease in T4:T1 consistent with the increased twitch depression it produced. As propofol alone did not produce train-of-four fade, we conclude that propofol must be potentiating vecuronium-induced twitch depression and vecuronium-induced decrease in train-of-four to the same degree.

**DISCUSSION**

After a typical induction dose of propofol 2 mg kg⁻¹, blood concentrations of propofol approach approximately 25–35 μmol litre⁻¹ (4–6 μg ml⁻¹), of which 98% is bound to plasma proteins [10–12]. In patients receiving continuous infusions of propofol up to 4.5 μg kg⁻¹ h⁻¹ for sedation in the intensive care unit, blood concentrations of propofol are still less than 10 μmol litre⁻¹ (1.7 μg ml⁻¹) [13]. Therapeutic concentrations of propofol had little effect on maximal diaphragmatic twitch tension. Twitch tension was inhibited only at much greater concentrations of propofol, with 50% inhibition occurring at 420 μmol litre⁻¹.
Our results also demonstrate that propofol 100 μmol litre⁻¹—a concentration that had no effect by itself—potentiated the non-depolarizing neuromuscular blocking drug vecuronium. In addition, train-of-four fade was detectable at smaller concentrations of vecuronium in the presence of propofol 100 μmol litre⁻¹.

The decrease in train-of-four produced by vecuronium is thought to be a presynaptic effect, perhaps reflecting decreased release of transmitter with successive stimulations [14]. T4:T1 was always directly proportional to the degree of twitch tension depression, regardless of whether or not twitch was depressed by vecuronium alone or in combination with propofol. The finding that T4:T1 was maintained as a function of the degree of block, rather than a function of vecuronium concentration, indicates that propofol also contributed to train-of-four fade and therefore potentiated both pre- and postsynaptic effects of the myoneural blocker.

On the basis of these experiments, we anticipate that neither muscle weakness nor potentiation of vecuronium-induced neuromuscular block should be of much concern at propofol concentrations seen clinically. Clinical studies of these potential problems have demonstrated little interaction between propofol and vecuronium [5-6]. Because of the large concentration required to potentiate vecuronium, it is unlikely that even a large dose of propofol, even in combination with other agents that displace it from plasma proteins, would result in propofol concentrations great enough to affect neuromuscular function.

REFERENCES