IN VITRO INVESTIGATION OF THE PRIMING PRINCIPLE FOR RAPID NEUROMUSCULAR BLOCK

R. J. STORELLA, J. JAFFE, E. MEHR AND H. ROSENBERG

The "priming principle" is used to hasten non-depolarizing neuromuscular block and facilitate intubation of the trachea by administering the drug in divided doses [1, 2]. A small fraction (10-15%) of the final dose of the drug, which should not cause significant weakness, is administered. Subsequently, after a priming interval (e.g. 3—5 min), the remainder of the blocker is administered. Several clinical reports have confirmed the effectiveness of the priming principle [3-7].

Although there is general agreement that priming can decrease the time to intubation, some investigators [8-11] have expressed concern regarding the safety of priming, suggesting that priming shortens intubation time only under conditions that compromise ventilation.

Schwarz and colleagues [2] suggested that priming decreases the margin of safety. Although this is commonly assumed to be the mechanism for priming [12], it has not been demonstrated in a quantitative manner. Black and colleagues [3] reported that the priming effect did not increase with the priming dose, a result which is not consistent with the mechanism of action proposed by Schwarz [2]. The involvement of prejunctional acetylcholine receptors also has been suggested [3, 5, 13, 14]. Thus the relative importance of the magnitude of the priming dose and the duration of the priming interval is uncertain [6,11,13,15,16].

The present in vitro study was designed to study these factors.

SUMMARY

The priming principle was investigated in the rat phrenic nerve–diaphragm preparation stimulated continuously at 0.2 Hz. Tubocurarine was added to the organ bath as either a single (non-primed) or a divided (primed) dose. Priming consisted of 15% or 20% of the final dose, with priming intervals of 5 or 10 min. Priming decreased significantly the time to 80% block and was associated with mild neuromuscular block. A simple model adequately predicted the time to 50% and 80% block, using the same diffusion constant for both primed and non-primed conditions. The onset of neuromuscular block, with and without priming, depended mostly upon the distribution of the drug to its site(s) of action.

MATERIALS AND METHODS

Male Sprague–Dawley rats were killed humanely and the left phrenic nerve–hemidiaphragm dissected and removed. The tissue was bathed in physiological saline solution (a modified Krebs containing (mmol litre\(^{-1}\)): NaCl 118; NaHCO\(_3\) 25; KCl 3.3; KH\(_2\)PO\(_4\) 1.1; CaCl\(_2\) 2.5; MgCl\(_2\) 0.9; glucose 11.1; choline chloride 0.01; pH = 7.4 at 37 °C) and aerated with carbon dioxide in oxygen. The left hemidiaphragm was cut into a strip approximately 5 mm wide, with the phrenic nerve inserting at its middle. Surgical silk suture (4-0) was tied around a small fragment of ribs and fixed to a post in the middle of a glass organ bath maintained at 37 °C containing 30 ml of physiological saline. The central tendon was ligated and connected to an isometric tension transducer (Grass FT-03). The phrenic nerve was ligated and drawn through a circular bipolar stimulating electrode which was suspended in the saline. The length of the preparation was adjusted to yield...
maximum tension to supramaximal stimulation of the phrenic nerve at 0.2 Hz (0.2-ms square wave).

After equilibration, tubocurarine was added in volumes of less than 200 μl. The drug treatment was divided into two periods. In the first period, a "priming dose" of 15 or 20% of the total dose, or no drug, was added. After a priming interval of 5 or 10 min, the remainder of the dose or the total dose was given. The final drug concentrations were approximately two or three times the ED₉₅.

Neuromuscular block was measured as the percent decrease in twitch tension from that immediately preceding the first period. The times to 50 and 80% block of contractile tension (Tᵢ₀ and Tᵢ₈₀) were measured from the beginning of the second period. One experiment was performed on each phrenic nerve-diaphragm preparation.

We examined the hypothesis that the onset of neuromuscular block, with and without priming, could be described by an application of Fick's law of diffusion. Rats were assigned randomly to one of six groups of eight animals. One group received tubocurarine in various concentrations, in order to determine a steady state concentration-response relationship. Both Tᵢ₀ and Tᵢ₈₀ were measured in the other five experimental groups to which tubocurarine was given. Three groups with a final concentration of 4.5 μmol litre⁻¹ were primed as follows: 20% for 5 min, 15% for 10 min and 20% for 10 min. Two groups were not primed (final concentration tubocurarine 4.5 and 3.0 μmol litre⁻¹). A typical recording of a primed preparation is shown in figure 1.

To test the diffusion hypothesis, we used the equation \(C = A - (A - C₀)e^{-kt}\), where \(C\) = concentration of drug at its site of action; \(A\) = drug concentration in bath; \(C₀\) = initial concentration at site of action; \(k\) = diffusion constant; and \(t\) = time. \(C\) was related to percent block by a dose-response relationship determined from stable degrees of block from single (non-primed) doses. \(k\) was calculated from non-primed \((C₀ = 0)\) preparations given tubocurarine 3.0 μmol litre⁻¹ and used in making predictions for the other groups. When predicting the onset of block following the administration of the second dose, \(C₀\) was estimated by calculating \(C\) at the end of the priming interval, using the Fick equation above.

Results are reported as mean (SEM). Comparisons were made by one-way analyses of variance followed by Dunnett's test, with \(P < 0.05\) regarded as significant.

**RESULTS**

The percent block produced by tubocurarine was determined for eight concentrations at steady state. The relationship between block and concentration was: \% block = (130 \cdot \log C) + 73 (r = 0.94). The calculated ED₉₅ was approximately 1.5 μmol litre⁻¹. In all preparations, tubocurarine both 3 and 4.5 μmol litre⁻¹ produced 100% neuromuscular block. The diffusion constant \((k)\) for tubocurarine was derived by solving the Fick equation for \(k\) using both the mean \(Tᵢ₀\) and the mean \(Tᵢ₈₀\) for tubocurarine 3.0 μmol litre⁻¹ and averaging the two values. This mean \(k\) \((k = 0.00086)\) was used to predict \(Tᵢ₀\) and \(Tᵢ₈₀\) for all tubocurarine-treated groups.

Priming significantly decreased onset time, as measured by \(Tᵢ₀\) and \(Tᵢ₈₀\) (tables I, II). The Fick model adequately predicted the onset of neuromuscular block. Table I shows that four of five predictions of \(Tᵢ₀\) were within 10% and 1 SEM of observed means. The least accurate prediction was within 20% and the 95% confidence interval.

![Fig. 1. Typical recording of isometric tension in a phrenic nerve-diaphragm preparation stimulated supramaximally at 0.2 Hz. A priming dose of 15% of the quantity of tubocurarine (tubo.) required for a final concentration of 4.5 μmol litre⁻¹ was given at the start of period I and the balance given after a 10-min priming interval. The recording periods are separated on the trace for clarity.](image-url)
TABLE I. Observed (mean (SEM)) and predicted times to 50° block for differing final concentration of tubocurarine, both primed and unprimed

<table>
<thead>
<tr>
<th>Final concn of tubocurarine (μmol litre⁻¹)</th>
<th>Time to 50° block</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed (s)</td>
<td>Predicted (s)</td>
</tr>
<tr>
<td>3.0</td>
<td>312 (38)</td>
<td>293</td>
</tr>
<tr>
<td>No prime</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>226 (19)</td>
<td>187</td>
</tr>
<tr>
<td>No prime</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primed</td>
<td>137 (22)</td>
<td>133</td>
</tr>
<tr>
<td>20°, 5 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15°, 10 min</td>
<td>119 (20)</td>
<td>115</td>
</tr>
<tr>
<td>20°, 10 min</td>
<td>95 (8.2)</td>
<td>90</td>
</tr>
</tbody>
</table>

TABLE II. Observed (mean (SEM)) and predicted times to 80° block for differing final concentration of tubocurarine, both primed and unprimed

<table>
<thead>
<tr>
<th>Final concn of tubocurarine (μmol litre⁻¹)</th>
<th>Time to 80° block</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed (s)</td>
<td>Predicted (s)</td>
</tr>
<tr>
<td>3.0</td>
<td>504 (66)</td>
<td>531</td>
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<tr>
<td>No prime</td>
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<td></td>
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<tr>
<td>4.5</td>
<td>356 (28)</td>
<td>326</td>
</tr>
<tr>
<td>No prime</td>
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<td></td>
</tr>
<tr>
<td>Primed</td>
<td>266 (39)</td>
<td>272</td>
</tr>
<tr>
<td>20°, 5 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15°, 10 min</td>
<td>232 (35)</td>
<td>253</td>
</tr>
<tr>
<td>20°, 10 min</td>
<td>213 (20)</td>
<td>228</td>
</tr>
</tbody>
</table>

DISCUSSION

Our data showed that priming accelerated the rate of onset of neuromuscular block in an in vitro rat preparation. Significant decreases in onset time were associated with a small degree of neuromuscular block produced by the priming dose, as has been demonstrated also in preliminary studies using vecuronium [17].

A simple model, based on Fick's law of diffusion, adequately described the onset of neuromuscular block (tables I, II) both with and without priming. Thus priming did not appear to change the effect of tubocurarine at its site(s) of action: These results are consistent with the hypothesis that the priming principle is a pharmacokinetic principle, and depends mostly on the distribution of a blocking drug to the neuromuscular junction. Although our results cannot exclude the possibility that prejunctional receptors are involved in acceleration of neuro-
muscular block produced by priming [3, 5, 6], the priming principle is explained reasonably well without recourse to such considerations.

The model of the priming principle has predictive value (fig. 3). It indicates that an effective way of increasing the rate of neuromuscular block is by increasing drug concentration. It predicts further that the rate constant for the onset of neuromuscular block is not affected by priming. Rather, the time to a given degree of neuromuscular block is decreased by priming. This may not be obvious if muscle force is measured, as transmission as determined from endplate potentials may be reduced beyond that which results in 100% block of induced twitch [18].

The model also indicates (fig. 3) how the effectiveness of priming is related to \( C_o \), the concentration of drug achieved at the neuromuscular junction at the end of the priming interval. It appears that small changes in \( C_o \) do not greatly decrease the time to 100% block of induced twitch. Thus changes in priming dose or interval which do not have a significant effect on \( C_o \) are not likely to change substantially the time to 100% block.

The clinical situation differs from our \textit{in vitro} system in several respects. The drug is distributed to more compartments \textit{in vivo} and is eliminated. The neuromuscular blocking drug may interact with a variety of other drugs used in the clinical setting and there are species differences in sensitivities to neuromuscular blocking drugs [19]. Thus the \textit{in vitro} model may not completely equate with the clinical setting.

However, the model accords with several clinical studies and permits generalizations. Small increases in priming doses did not substantially increase the effectiveness of priming in clinical studies [6,15]. The model indicates that the decreased onset time of small increases in priming dose or interval would be small and would be obscured by the variable responsiveness of nerve-muscle systems to non-depolarizing blocking drugs [20]. Donati and colleagues [21] came to a similar conclusion using a model of human neuromuscular block.

We conclude that it is unlikely that any combination of priming dose and duration of priming interval can be found that substantially reduces weakness, and thus respiratory impairment, while retaining an appreciably accelerated onset of complete block. However, techniques that potentiate the block when the induction dose is administered (for example synergistic combinations of non-depolarizing blocking drugs [21]) would be expected to improve the priming principle.

ACKNOWLEDGEMENTS

This work was supported in part by the Biomedical Research Support Grant No. 2 S07 RR50413 and the Hahnemann Anesthesia Research Foundation.

REFERENCES

1. Gergis SD, Sokoll MD, Mehta M, Kemmotsu O, Rudd GD. Intubation conditions after atracurium and


