The Influence of Respiratory-induced Acid-base Changes on the Action of Non-depolarizing Muscle Relaxants in Rats

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The influence of respiratory-induced acid-base changes on the action of non-depolarizing muscle relaxants was investigated using the rat phrenic nerve-hemidiaphragm preparation. Changes in pH were induced by changes in the CO₂ concentration aerating Krebs’ solution. In the absence of muscle relaxants, an increase in CO₂ from 5% to 7.5% decreased (P < 0.01) indirectly elicited twitch tension by 5.4 ± 0.7% (mean ± SEM), while a decrease in CO₂ from 5% to 2.5% increased (P < 0.01) twitch tension by 2.3 ± 0.7%. With a change in CO₂ from 2.5% to 7.5%, partial neuromuscular blockade produced by d-Tc or vecuronium was augmented (P < 0.01), while that produced by metocurine, pancuronium, or alcuronium was reduced (P > 0.01). With the change in CO₂ from 7.5% to 2.5%, the neuromuscular blockade produced by d-Tc or vecuronium was reduced (P < 0.01), while that produced by metocurine, pancuronium, or alcuronium was augmented (P < 0.01). Dose-response study showed that 2.5% CO₂ shifted the dose-response curves for d-Tc and vecuronium to the right (P < 0.01) from those with 5% CO₂, whereas 7.5% CO₂ shifted them to the left (P < 0.05). In contrast, neither 2.5% CO₂ nor 7.5% CO₂ significantly shifted the dose-response curves for metocurine or pancuronium from those with 5% CO₂. Their dose-response curves with 2.5% CO₂ were to the left, instead of to the right, of those with 7.5% CO₂ (P < 0.01). These discrepancies in the response to respiratory-induced pH changes may be attributed to the difference in chemical structure of these muscle relaxants, that is, the monoquaternary nature of d-tubocurarine and vecuronium versus the bisquaternary structures of the other compounds. (Key words: Acid-base equilibrium: acidosis, respiratory, alkalosis, respiratory. Neuromuscular relaxants: alcuronium; d-tubocurarine; metocurine; pancuronium; vecuronium.)

The influence of respiratory-induced acid-base changes on the neuromuscular effects of nondepolarizing muscle relaxants (MRs) has been investigated extensively.1-15 Respiratory acidosis has been found either to augment or not to alter neuromuscular blockade produced by d-tubocurarine (d-Tc).1-2,4-8,12 pancuronium,9,11,13,15 or vecuronium,14,15 while it has been found either to reduce or not to alter metocurine,2,4,8 or gallamine-induced1,8 blockade. Respiratory alkalosis has been found either to reduce or not to alter d-Tc-4,8,12 or vecuronium-induced14,15 blockade, while it has been found either to augment or not to alter metocurine-,2,4,8 pancuronium-,11,13,15 or gallamine-induced8 blockade. The varying results have been attributed to experimental design and to species differences. Additionally, most of the previous experiments have been performed on either anesthetized animals or humans. In such in vivo experiments, not only changes in the pharmacodynamics of MRs but also those in their pharmacokinetics, e.g., alterations in blood flow, have to be considered.

The purpose of this study was to determine in in vitro experiments, in which many variables of in vivo studies can be eliminated, the influence of respiratory-induced pH changes on neuromuscular transmission both in the presence and in the absence of nondepolarizing MRs.

Materials and Methods

The experiments were carried out using the rat phrenic nerve-hemidiaphragm preparation.16 Male Sprague-Dawley rats of 300–350 g body weight were decapitated, and the hemidiaphragms were dissected along with their phrenic nerves. The preparations were suspended in double-jacketed organ baths of 100 ml volume filled with modified Krebs’ solution (NaCl 113.0; KCl 4.7; CaCl₂ 1.4; KH₂PO₄ 1.2; MgSO₄ 1.2; NaHCO₃ 25.0; Glucose 1.5 mM).17 The solution in the bath was maintained at 37°C by circulating water from a thermostatically controlled water bath, and aerated with a mixture of CO₂ and O₂. The concentration of CO₂ was controlled with flowmeters (Shimazu 1203), and the pH of the solution aerated with each concentration of CO₂ was determined with a pH meter (Orion 611).

In preliminary experiments, the pH of the modified Krebs’ solution aerated with 2.5%, 5%, or 7.5% CO₂ in O₂ was 7.59 ± 0.01, 7.37 ± 0.004, and 7.21 ± 0.01 (mean ± SEM, n = 5), respectively.

Supramaximal square wave stimuli of 10–15 V and 0.2 msec duration were applied to the phrenic nerves every 10 s through a bipolar platinum electrode. Indirectly elicited twitch tension was quantitated by TB-6111T force displacement transducers (Nihon Kohden Co., Tokyo, Japan) and continuously recorded. After twitch tension became stabilized with 5% CO₂ in 95% O₂, three series of experiments were performed.

First, the influence of changes in CO₂ on indirectly elicited twitch tension in the absence of MRs was observed. The concentration of CO₂ in O₂ was changed...
from 5% to either 2.5% or 7.5%, and the changes in twitch tension were observed.

Second, the influence of changes in CO₂ on partial neuromuscular blockade produced by d-Tc, vecuronium, metocurine, pancuronium, or alcuronium was investigated. After stable twitch tension was obtained either with 2.5% CO₂ or with 7.5% CO₂, 0.82 μM of d-Tc, 5.6 μM of vecuronium, 0.22 μM of metocurine, 3.3 μM of pancuronium, or 1.2 μM of alcuronium was added to the bath to produce about a 70% neuromuscular blockade. Forty minutes later, the concentration of CO₂ was changed either from 2.5% to 7.5% or from 7.5% to 2.5%, and the changes in twitch tension were observed. At the end of each experiment, MR was washed out and nearly complete recovery of twitch tension was noted.

Third, to determine the influence of changes in CO₂ on the potency of MRs more quantitatively, log dose-response curves for d-Tc, vecuronium, metocurine, and pancuronium were constructed at each of the three pH levels by assessing neuromuscular blockade at three dose levels of each MR at each pH. The neuromuscular blockade produced by addition of each of the three doses of one of the MRs was quantitated using five different preparations. Consequently, a dose-response regression line for the MR at each of the three pH levels was determined from 15 points with a least squares method. To rule out the influence of fatigue and that of previously administered MRs, each preparation was used for only one experiment. The data in the first and second experiments were tested for statistical significance using paired Student's t test. The dose response curves in the third experiment were compared between groups by analysis of variance and Bonferroni t test. Differences at the P < 0.05 level were considered statistically significant.

Results

The Influence of Changes in CO₂ on Indirectly Elicited Twitch Tension

When the concentration of CO₂ was increased from 5% to 7.5%, twitch tension decreased (P < 0.01) by 5.4 ± 0.7% (mean ± SEM, n = 5) from the control. When CO₂ was decreased from 5% to 2.5%, twitch tension increased (P < 0.01) by 2.3 ± 0.7% from the control (n = 5).

The Influence of Changes in CO₂ on the Neuromuscular Blockade Produced by d-Tc, Vecuronium, Metocurine, Pancuronium, or Alcuronium

When the concentration of CO₂ was increased from 2.5% to 7.5%, twitch tension in the presence of d-Tc or vecuronium decreased (P < 0.01) by 29.4 ± 1.6% (n = 4) and 18.1 ± 1.5% (n = 4), respectively. In contrast, twitch tension in the presence of metocurine, pancuronium, or alcuronium increased (P < 0.01) by 3.7 ± 1.3% (n = 4), 9.9 ± 1.6% (n = 4), and 9.4 ± 2.0% (n = 4), respectively (Fig. 1A). When the concentration of CO₂ was decreased from 7.5% to 2.5%, twitch tension in the presence of d-Tc or vecuronium increased (P < 0.01) by 8.3 ± 2.2% (n = 4) and 16.3 ± 0.9% (n = 4), while twitch tension in the presence of metocurine, pancuronium, or alcuronium decreased (P < 0.01) by 6.7 ± 1.5% (n = 4), 14.1
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The Influence of Changes in CO₂ on the Potency of d-Tc, Vecuronium, Metocurine, and Pancuronium (Dose-response Study)

The dose-response curves for d-Tc, vecuronium, metocurine, and pancuronium are shown in figure 2A–D, and their ED₅₀ values in table 1 and figure 3. Two and a half percent CO₂ shifted the dose-response curves for d-Tc and vecuronium to the right (P < 0.01) in parallel from those with 5% CO₂, while 7.5% CO₂ shifted them to the left (P < 0.05).

In contrast, although neither 2.5% CO₂ or 7.5% CO₂ significantly shifted the dose-response curves for metocurine or pancuronium from those with 5% CO₂, the comparison of the 2.5% CO₂ group with the 7.5% CO₂ group did show a statistically significant difference, indicating the curves for metocurine and pancuronium with 2.5% CO₂ was to the left, instead of to the right, of those with 7.5% CO₂ (P < 0.01).

As shown in table 1 and figure 3, the ED₅₀ values of d-Tc and vecuronium were higher for the preparations exposed to 2.5% CO₂, than for those exposed to 7.5% CO₂. In contrast, the ED₅₀ values of metocurine and pancuronium were higher in the presence of 7.5% CO₂.

### Table 1. The Influence of Changes in CO₂ on the ED₅₀ of d-Tc, Vecuronium, Metocurine, and Pancuronium.

<table>
<thead>
<tr>
<th>Compound</th>
<th>5% CO₂</th>
<th>7.5% CO₂</th>
<th>2.5% CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-Tc</td>
<td>0.647</td>
<td>0.599*</td>
<td>0.744†</td>
</tr>
<tr>
<td></td>
<td>(0.617–0.678)</td>
<td>(0.577–0.622)</td>
<td>(0.680–0.795)</td>
</tr>
<tr>
<td>Vecuronium</td>
<td>4.45</td>
<td>4.03*</td>
<td>5.92†</td>
</tr>
<tr>
<td>Metocurine</td>
<td>0.174</td>
<td>0.199</td>
<td>0.165‡</td>
</tr>
<tr>
<td></td>
<td>(0.160–0.189)</td>
<td>(0.178–0.221)</td>
<td>(0.152–0.179)</td>
</tr>
<tr>
<td>Pancuronium</td>
<td>5.09†</td>
<td>3.21</td>
<td>2.81‡</td>
</tr>
<tr>
<td></td>
<td>(2.86–5.35)</td>
<td>(2.93–3.51)</td>
<td>(2.70–2.92)</td>
</tr>
</tbody>
</table>

Each ED₅₀ value was obtained from 15 experiments. Figures in parenthesis represent 95% confidence limits.

* and † indicate significant difference from ED₅₀ values with 5% CO₂ at P < 0.05 and P < 0.01 levels, respectively.
‡ Indicates significant difference from ED₅₀ values with 7.5% CO₂ at P < 0.01 level.
than they were with 2.5% CO₂, although they were affected by the changes in CO₂ to a lesser degree than in the case of d-Tc and vecuronium.

**Discussion**

It has been reported that not only the potency of MRs, but also indirectly elicited muscle contraction itself, can be affected by respiratory-induced pH changes.₁⁻³, 6,12, 14-18, 18-19 The first finding of our study was that indirectly elicited contractions of the diaphragm were increased with a higher pH and decreased with a lower pH. These findings are similar to the results of previous reports.

In the second part of this study, the influence of respiratory-induced pH changes on the action of various MRs was measured and correlated under controlled conditions. As a result, it was found that the influence of respiratory-induced pH changes on the potency of d-Tc and vecuronium was very different from that on the potency of metocurine, pancuronium, and alcuronium. The neuromuscular blocking action of d-Tc and vecuronium was potentiated by 7.5% CO₂, while it was antagonized by 2.5% CO₂. In contrast, although the action of metocurine and pancuronium was affected by pH changes to a lesser degree, significant opposite changes did occur between the 2.5% CO₂ and 7.5% CO₂ groups. The action of metocurine and pancuronium was greater with 2.5% CO₂ than with 7.5% CO₂. The same tendency was also observed with alcuronium.

What is the mechanism of these differences? There is a general agreement among previous reports that the action of d-Tc is potentiated by respiratory acidosis, while it is antagonized by respiratory alkalosis. ¹⁻⁴⁻⁶, ⁸ Kalow ⁹ suggested that the change in the potency of d-Tc may be attributed to an alteration in the degree of ionization of two hydroxy groups in d-Tc molecules. An increase in the potency of d-Tc in acidosis was explained by a reduction in the degree of ionization. However, this theory was challenged by Katz et al. ²¹ on the grounds that the change in ionization in a clinical pH range of ±0.4 pH units would be insufficient to account for any changes in the potency of d-Tc. In 1970, the chemical structure of d-Tc was revised by Everett et al. ²² They pointed out that d-Tc is a monoquaternary ammonium compound, which had only one quaternary ammonium group at a physiological pH. The charge density on the other tertiary ammonium group would be increased by combining with an H⁺ ion at a lower pH, and pseudo-bisquaternary compounds would be formed. Feldman ²³ suggested that these pseudo-bisquaternary compounds, which have a more positive charge than original monoquaternary form, possess a greater affinity for, and, also, a greater specificity for, anionic acetylcholine receptors. Therefore, it would be more reactive in acidosis. This hypothesis is supported by the finding of Durant et al. ²⁴ that the neuromuscular blocking potency of N-methyl bisquaternary analogue of d-Tc was greater than that of the original compound in both in vitro and in vivo preparations of cats. Our findings with d-Tc and its O, O, N-trimethyl bisquaternary analogue, metocurine, also gave support to it.

Vecuronium is a monoquaternary nondepolarizing MR, ²⁵ and was less potent than its bisquaternary analogue, pancuronium, in rats. Similar to d-Tc, its neuromuscular blocking action was potentiated at a lower pH and antagonized at a higher pH. These findings suggest that alterations in the potency of monoquaternary compounds, e.g., d-Tc and vecuronium, may depend on the degree of pseudo-bisquaternary compound formation at different pH levels.

Our results on d-Tc and vecuronium partially conflict with those of previous studies.₁², ¹⁴⁻¹⁵ Miller et al. ¹²

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using 90% neuromuscular blockade produced by a continuous infusion of d-Tc, reported that neither respiratory acidosis or alkalosis altered the infusion rate of d-Tc required to maintain the blockade. In view of the relationship between concentration of MRs and neuromuscular blockade (the sigmoid shaped dose-response curve), the observation on 90% neuromuscular blockade might not be sensitive enough to detect pH-induced changes in the action of d-Tc. Funk et al.\textsuperscript{14} and Gencarelli et al.,\textsuperscript{15} using 50% neuromuscular blockade produced by a continuous infusion of vecuronium, reported that respiratory acidosis potentiated the action of vecuronium, while respiratory alkalosis did not alter it. They attributed the above observation, in part, to an increased rate of vecuronium metabolism by alkaline hydrolysis in alkalotic states and greater molecular stability in acidosis. In the experiment by Funk et al., the degree of pH changes induced during respiratory alkalosis was much less than that induced during respiratory acidosis (0.17 pH units in alkalosis versus 0.39 pH units in acidosis), and this difference might have affected their results. In the experiment by Gencarelli et al., respiratory acidosis was induced by both a reduction of ventilation and an inhalation of 5–10% CO\textsubscript{2}, while respiratory alkalosis was induced by hyperventilation during halothane anesthesia. Although halothane end-tidal concentration was reported to be maintained relatively constant, it is conceivable that the potentiating effect of halothane on the action of MRs might have obscured the opposite changes in vecuronium-induced neuromuscular blockade by respiratory alkalosis.

In contrast, the influence of respiratory-induced pH changes on the potency of metocurine, pancuronium, and alcuronium have not been clearly defined.\textsuperscript{2–4,8–9,11–13,15} Since most of these previous studies were designed to determine the effect of pH changes on pre-existing neuromuscular blockade produced by MRs, pH-induced changes in muscle contraction itself might have affected their results. The changes in pharmacokinetics in \textit{in vivo} situations has to be considered as well. In this study, we found that the action of these bisquaternary MRs was greater at a higher pH than at a lower pH, although it was affected by pH changes to a lesser degree than in the case of monoquaternary d-Tc or vecuronium. The effect on muscle contraction itself was eliminated in the present dose-response study. This opposite effect of pH changes on the potency of bisquaternary MRs may be explained by the alteration of the binding properties of the receptor sites. At a higher pH, more anionic receptor sites would be expected.\textsuperscript{23} Since a change in the charge density of the quaternary ammonium group in bisquaternary MR is not probable, the influence of changes in receptor properties may become prominent. In the case of monoquaternary MRs, the changes in MR affinity are probably so influential that the effect on receptor affinity may not be detected. Therefore, we assumed that a basis for these observations might be due to the monoquaternary nature of d-Tc and vecuronium \textit{versus} bisquaternary structures of metocurine, pancuronium, and alcuronium. Changes in the action of monoquaternary compounds may be attributed to changes in their affinity to the receptor, which depend on the degree of pseudo-bisquaternary compound formation at different pH levels. In the case of bisquaternary MRs, alterations in the receptor affinity caused by the changes in their ionization may be responsible. In view of this assumption, it is likely that the influence of metabolic-induced pH changes may be similar to that of respiratory-induced changes. However, there have been several reports\textsuperscript{2–4,12–15} which indicate that the influence of respiratory-induced acid-base changes is different from that of metabolic-induced acid-base changes in both direction and intensity. Payne\textsuperscript{13} reported that the neuromuscular blocking action of d-Tc in cats were potentiated with CO\textsubscript{2} inhalation, while antagonized with hydrochloric acid infusion and potentiated with sodium bicarbonate infusion. Discrepancies between respiratory- and metabolic-induced pH changes in their effects on the action of d-Tc,\textsuperscript{12} pancuronium,\textsuperscript{15} and vecuronium\textsuperscript{14} were also reported. Since pH changes in this study were induced by changes in CO\textsubscript{2} aerating modified Krebs' solution, only the influence of respiratory-induced acid-base changes was investigated. The influence of metabolic-induced acid-base changes has yet to be determined under similar experimental conditions.

Hughes\textsuperscript{8} pointed out that the effect of pH changes depend on whether it was produced before the MRs had been given or during recovery. He reported that the effect of pH changes induced during recovery was different from their known effects. In contrast, Gencarelli et al.\textsuperscript{15} reported that pre-muscle relaxant administration-induced hypercapnia or hypocapnia had no effect on a subsequent neuromuscular blockade from pancuronium or vecuronium. Furthermore, Funk et al.\textsuperscript{14} reported that the effect of pH changes on pre-existing vecuronium-induced blockade was similar to that observed in the cumulative dose-response study, in which pH changes had been induced before MRs were given. In our study, pH changes were induced in both ways under \textit{in vitro} conditions, and their effects were found to be similar. At present, we do not have any explanation for these variances.

In conclusion, indirectly elicited contractions of the diaphragm of rats in the absence of MRs were decreased by a change to a lower pH and increased by a change to a higher pH, both induced by changes in the concentration of CO\textsubscript{2}. As for the alteration in the po-
tency of nondepolarizing MRs in respiratory-induced acid-base changes, the action of d-Tc and vecuronium was potentiated at a lower pH and antagonized at a higher pH. In contrast, although the action of metocu-
rine, pancuronium, and alcuronium was affected by res-
piratory-induced pH changes to a lesser degree, their action was greater at a higher pH than at a lower pH. A basis for these observations may be the monoquaternary nature of vecuronium and d-Tc versus the bisqua-
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