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Neuromuscular Effects of Respiratory and Metabolic Acid–Base Changes In Vitro *With and Without Nondepolarizing Muscle Relaxants*

Kazumi Ono, M.D., Osamu Nagano, M.D.,† Yoshio Ohta, M.D.,* Futami Kosaka, M.D.‡*

The effects of metabolic (bicarbonate, [HCO_3]) and respiratory (carbon dioxide, P_{CO_2}) acid–base changes on indirectly elicited twitch tension with and without nondepolarizing neuromuscular blocking agents were compared in a rat phrenic nerve–hemidiaphragm preparation. Ionized calcium [Ca^{2+}] and magnesium [Mg^{2+}] concentrations in the modified Krebs' solution were measured and kept constant. Likewise, twitch was altered when *p*H changes were produced by altering either P_{CO_2} or [HCO_3]. Decreasing *p*H either by increasing P_{CO_2} or by decreasing [HCO_3] significantly decreased ($P < 0.01$) twitch, by 9.5 ± 0.6 (SEM, n = 8) and 10.6 ± 1.5%, respectively. Increasing *p*H by decreasing P_{CO_2} or by increasing [HCO_3] significantly increased ($P < 0.01$) twitch, by 5.6 ± 0.9 and 7.9 ± 0.6%, respectively. After a partial depression of twitch by nondepolarizing neuromuscular blocking agents, the effects of P_{CO_2} and [HCO_3] changes were again assessed. Decreasing *p*H by increasing P_{CO_2} or by decreasing [HCO_3] intensified d-tubocurarine (dTc) (28.2 ± 1.6 and 32.0 ± 2.9%, respectively) and vecuronium (23.0 ± 1.4 and 36.8 ± 3.2%, respectively) block, whereas it reversed metocurine (1.2 ± 2.2% NS and 2.9 ± 1.3%, respectively) and pancuronium (8.3 ± 1.5 and 11.5 ± 3.0%, respectively) block. Conversely, increasing *p*H by decreasing P_{CO_2} or by increasing [HCO_3] antagonized dTc (12.8 ± 2.2 and 13.6 ± 1.8%, respectively) and vecuronium (25.3 ± 1.7 and 25.0 ± 3.0%, respectively) block, whereas it potentiated metocurine (4.2 ± 0.6 and 8.0 ± 1.1%, respectively) and pancuronium (11.0 ± 1.2 and 17.5 ± 2.0%, respectively) block. Except where indicated, all changes in block described above were statistically significant. These findings suggest that neither CO_2 nor [HCO_3] specifically has an action on the neuromuscular junction, but rather, that changes in *p*H were responsible for the observed changes in twitch. In addition, treatment with monoquaternary neuromuscular blocking (dTc and vecuronium) agents results in a different response to acid–base changes than that after bisquaternary (metocurine and pancuronium) agents. (Key words: Neuromuscular blocking drugs: d-tubocurarine; vecuronium; metocurine; pancuronium. Acid–base equilibrium: respiratory; metabolic.)

PREVIOUS PUBLICATIONS have provided conflicting information concerning the effects of respiratory and metabolic acid–base changes on preexisting partial nondepolarizing neuromuscular block. For example, Payne[1] reported that the *in vivo* action of d-tubocurarine (dTc) in the cat was potentiated by carbon dioxide (CO_2) inhalation and antagonized by hydrochloric acid (HCl) infusion. Miller *et al.*,[2] using a constant infusion of dTc, found that changes in CO_2 did not alter partial dTc block, but that HCl-induced acidosis antagonized and bicarbonate (HCO_3)- or carbonate-induced alkalosis potentiated the dTc block. In contrast, there have been several conflicting studies[3–6] that have indicated that effects of metabolic acid–base changes on dTc are similar to the known effects of respiratory acid–base changes. As with dTc, previous publications have provided conflicting information concerning the effects of respiratory and metabolic acid–base changes on preexisting partial nondepolarizing neuromuscular block caused by metocurine[1,3–6] and by pancuronium.[7,8]

In our previous dose–response study with CO_2,[9] we demonstrated that the neuromuscular blocking potencies of monoquaternary dTc and vecuronium were less than those of their bisquaternary analogues, metocurine and pancuronium, respectively. In addition, the blocking potency of both dTc and vecuronium were augmented by increasing the CO_2 and reduced by decreasing the CO_2. We hypothesized that these CO_2-induced changes in the potencies of monoquaternary compounds might be attributed to a *p*H-dependent pseudo-bisquaternary compound formation and a resulting change in the affinity to anionic acetylcholine receptors. If this hypothesis is true, it is likely that the same is true for metabolic acid–base changes.

The purpose of the current study was to compare the neuromuscular effects of respiratory and metabolic acid–base changes with and without nondepolarizing neuromuscular blocking agents, with the use of phrenic nerve–hemidiaphragm preparations of the rat. *In vitro* preparations were chosen to eliminate pharmacokinetic variables. Respiratory and metabolic acid–base changes were induced by changes in P_{CO_2} and [HCO_3]. When [HCO_3] was altered in the modified Krebs' solution, changes in ionized calcium [Ca^{2+}] and magnesium [Mg^{2+}] concentrations accompanied *p*H changes. To separate the effects of these [Ca^{2+}] and [Mg^{2+}] changes from those of *p*H changes, experiments were done with [Ca^{2+}] and [Mg^{2+}] kept constant.

* Assistant Professor of Anesthesiology and Resuscitology.

† Research Fellow.

‡ Professor and Chairman of Anesthesiology and Resuscitology.

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Address reprint requests to Dr. Ono: Department of Anesthesiology and Resuscitology, Okayama University Medical School, 2-5-1 Shikata-Cho, Okayama, 700, Japan.

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Materials and Methods

In accordance with the Institutional Animal Care Committee standards, male Sprague-Dawley rats weighing 275–325 g were decapitated, and each hemidiaphragm removed with its attached phrenic nerve. The dissected phrenic nerve–hemidiaphragm preparations[10] were then mounted: the muscular portion was fixed with metal hooks to the bottom of an organ bath, and the tendon was attached to TB-611T force displacement transducers (Nihon Kohden Co., Tokyo, Japan) above the bath. The double-jacketed organ bath had a volume of 100 ml and was filled with modified Krebs' solution (see table 1 for electrolyte compositions).[11] Solution temperature was maintained at 37° C by circulating water from a thermostatically controlled water bath and bubbled with a mixture of 95% O_2 and 5% CO_2 through a needle at the bottom of the bath. To promote equilibrium of CO_2 in the solution, the top of the bath was covered with a plastic plate with a slit (fig. 1). Each preparation was stimulated *via* the phrenic nerve with 0.1-Hz supramaximal square wave impulses of 0.2 ms duration, and elicited twitch tension was continuously recorded. Optimal pretension was determined by gradually increasing the resting tension until further stretching of the preparation did not cause any additional increase in tension output.

According to the Henderson-Hasselbach equation, the *p*H of the modified Krebs' solution depends on the ratio of the P_{CO_2} and $[HCO_3]$. P_{CO_2} and $[HCO_3]$ were manipulated to produce respiratory and metabolic acid–base changes *in vitro*, respectively. P_{CO_2} was changed by varying CO_2 concentration of the bubbling gas to 2.5 or 9% with flowmeters (Shimazu 1203, Kyoto, Japan). To create *p*H changes similar to those caused by 2.5 and 9% CO_2, $[HCO_3]$ was changed by replacing the bathing fluid with the modified Krebs' solution containing 50 and 13.5 mM HCO_3, respectively.

In the preliminary experiments, $[Ca^{2+}]$ and $[Mg^{2+}]$ in the modified Krebs' solution were measured after manipulation of P_{CO_2} or $[HCO_3]$ since these electrolytes change in acutely induced acid–base changes. *p*H and P_{CO_2} of the solution were also measured (ABL 4/0 Radiometer, Copenhagen). Measurements of $[Ca^{2+}]$ and $[Mg^{2+}]$ were made potentiometrically at 37° C with a Ca^{2+}-sensitive electrode (Orion 9320) and divalent cation-sensitive electrode (Orion 9332) connected to an ion analyzer (Orion EA920). $[Mg^{2+}]$ was calculated as the difference between [divalent cation] and $[Ca^{2+}]$. The coefficient of variation of the assay was less than 10%. As expected, the *p*H of the solutions containing 25 mM HCO_3 and bubbled with 2.5 and 9% CO_2 were similar to the *p*H of the solutions containing 50 and 13.5 mM HCO_3 bubbled with 5% CO_2, respectively (table 2). $[Ca^{2+}]$ and $[Mg^{2+}]$ of the solution of 25 mM HCO_3 bubbled with 5% CO_2 were 1.15 ± 0.01 and 0.76 ± 0.01, respectively. Changes in CO_2 to 2.5 or 9% did not alter $[Ca^{2+}]$ or $[Mg^{2+}]$. In contrast, changes in $[HCO_3]$ to 13.5 and 50 mM did alter $[Ca^{2+}]$ to 1.21 ± 0.01 and 0.99 ± 0.01, respectively. $[Mg^{2+}]$ was also altered to 0.79 ± 0.02 and 0.63 ± 0.01, respectively. To separate the effect of these $[Ca^{2+}]$ and $[Mg^{2+}]$ changes from that of the *p*H changes, $[Ca^{2+}]$ and $[Mg^{2+}]$ of the replacing solutions were kept constant at 1.15 and 0.76 mM, respectively, with adjustment of total Ca and Mg. Osmolarity of the replacing solution was also kept constant by reciprocal changes in chloride and glucose concentration. Electrolyte composition of these replacing solutions were shown in table 1.

With the preparations and the *p*H manipulations described above, the effects of respiratory and metabolic *p*H changes were compared under the same conditions. The effect of changes in P_{CO_2} and $[HCO_3]$ on twitch tension was assessed without neuromuscular blocking agents. After control twitch tension of the hemidiaphragm was ob-

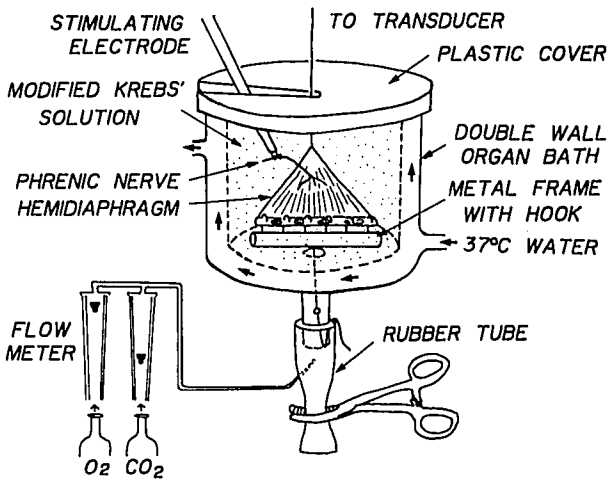

FIG. 1. Phrenic nerve–hemidiaphragm preparation of rat.

TABLE 1. Electrolyte Compositions of the Modified Krebs' Solutions (mM)

Electrolyte	Modified Krebs' Solutions		
	$[HCO_3]$-Decreased	Control	$[HCO_3]$-Increased
HCO_3	13.5	25.0	50.0
Na	138.0	138.0	138.0
K	5.9	5.9	5.9
Ca	1.3	1.4	1.65
Mg	0.85	0.9	1.08
Cl	131.8	120.5	96.0
H_2PO_4	1.2	1.2	1.2
SO_4	0.85	0.9	1.08
Glucose	11.9	11.5	10.4

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TABLE 2. pH and P_{CO_2} of the Modified Krebs' Solution with Manipulations of P_{CO_2} or $[HCO_3]$

$[HCO_3]$ (mM)	CO_2 (%)	pH	P_{CO_2} (mmHg)
25.0	5.0	7.42 ± 0.01	39.1 ± 1.0
25.0	2.5	7.66 ± 0.02	23.9 ± 1.0
25.0	9.0	7.18 ± 0.01	66.0 ± 1.9
13.5	5.0	7.19 ± 0.01	36.9 ± 0.8
50.0	5.0	7.72 ± 0.01	38.8 ± 0.8

Each value is mean ± SEM, n = 10.

tained in the 5% CO_2 and 25 mM HCO_3 solution, eight preparations were exposed to the 2.5% CO_2 and 25 mM HCO_3 solution for 80 min. Thereafter, these preparations were exposed to the 9% CO_2 and 25 mM HCO_3 solution for 30 min, and the amount of change in twitch tension associated with the P_{CO_2}-induced decrease in *p*H was determined. Eight other preparations were initially exposed to the 9% CO_2 and 25 mM HCO_3 solution for 80 min and then exposed to the 2.5% CO_2 and 25 mM HCO_3 solution. The amount of change in twitch associated with the P_{CO_2}-induced increase in *p*H was determined 30 min later. To investigate HCO_3-induced *p*H changes similar to those caused by P_{CO_2}, preparations were initially exposed to either 5% CO_2 and 50 mM HCO_3 solution (n = 8) or 5% CO_2 and 13.5 mM HCO_3 solution (n = 8). Eighty minutes later, the 50 and 13.5 mM HCO_3 solutions were replaced by the 13.5 and 50 mM HCO_3 solutions, respectively, both aerated with 5% CO_2. The replacing solutions had been equilibrated with 5% CO_2 and solution temperature had been kept at 37°,C immediately before replacement. The amounts of change in twitch tension associated with these HCO_3-induced *p*H changes were determined 30 min later.

The effects of changes in P_{CO_2} and $[HCO_3]$ were then assessed after a partial depression of twitch tension by nondepolarizing neuromuscular blocking agents. In these experiments, after stable control twitch tension was obtained in the 5% CO_2 and 25 mM HCO_3 solution, preparations were exposed to one of the following solutions: 2.5% CO_2 and 25 mM HCO_3, 9% CO_2 and 25 mM HCO_3, 5% CO_2 and 50 mM HCO_3, or 5% CO_2 and 13.5 mM HCO_3, for 20 min. Then a partial depression in twitch tension was produced by adding a preselected dose of dTc (0.65 μM), vecuronium (4.45 μM), metocurine (0.17 μM), or pancuronium (3.09 μM). Each dose given was the ED_{50} value of the blocking agent according to the previous dose–response study[9] in the 5% CO_2 and 25 mM HCO_3 solution. Sixty minutes later, changes were made in either CO_2 (from 2.5 to 9% or from 9 to 2.5%) or $[HCO_3]$ (from 50 to 13.5 mM or from 13.5 to 50 mM) in the same way as the experiments without the blocking agents. This time, however, the replacing 13.5 and 50 mM HCO_3 solutions contained the same concentration of the neuromuscular blocking agents as before. The amounts of change in twitch tension were determined 30 min later. As in the case without the blocking agents, eight different preparations were used for each experiment.

Statistical analyses were performed to evaluate the amount of change in twitch after P_{CO_2} or $[HCO_3]$ manipulations. In each preparation with or without neuromuscular blocking agent, the value of twitch tension prior to the manipulation of P_{CO_2} or $[HCO_3]$ was compared with that after the manipulation using the paired Student's t test. For comparison of the value of twitch after decrease or increase of P_{CO_2} or $[HCO_3]$, analysis of variance was used. Differences in the amounts of change in twitch between those with each neuromuscular blocking agent and those without the blocking agents were tested by analysis of variance and the Bonferroni t test. Comparison of the amounts of change in twitch was also made between those induced by P_{CO_2} manipulation and those after HCO_3 replacement, which created similar *p*H changes with analysis of variance. Differences were considered statistically significant if P was <0.05.

Results

Values of twitch tension prior to and after manipulations of P_{CO_2} or $[HCO_3]$ and the resulting amounts of change in twitch are summarized in table 3 and table 4. Each value was expressed as percentage of control twitch tension in the 5% CO_2 and 25 mM HCO_3 solution. As shown in figure 2, twitch tension stabilized rapidly after exposure to a different P_{CO_2} without neuromuscular blocking agents. In contrast, twitch changed slowly over a long period of time after a replacement of HCO_3. Decreasing *p*H by either increasing P_{CO_2} or decreasing $[HCO_3]$ caused a significant decrease ($P < 0.01$, paired t test) in twitch without the blocking agents (see also table 3). Increasing *p*H by decreasing P_{CO_2} or increasing $[HCO_3]$ caused a significant increase ($P < 0.01$) in twitch (see also table 4). There was no significant difference in the amounts of change in twitch (values in % Change column in tables 3 and 4) between those after P_{CO_2} manipulation and those after HCO_3 replacement, which created a similar *p*H change (analysis of variance). The values of twitch tension when a decrease of $[HCO_3]$ was induced were not the same as those when an increase was induced ($P < 0.01$, analysis of variance). This was not apparent when changes were made in P_{CO_2}. In addition, because of the experimental design, a measurement of twitch prior to the $[HCO_3]$ change was made after exposure to each $[HCO_3]$ solution for 80 min, whereas that after the change was made after a 30-min exposure. Considering the slow and sustained effects of the changes in $[HCO_3]$, the different

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TABLE 3. Effects of Decreasing *p*H Caused by Increasing CO_2 or Decreasing [HCO_3] on Twitch Tension with and without *d*Tc, Vecuronium, Metocurine, and Pancuronium

	Values and Amounts of Change in Twitch Tension					
CO_2 or [HCO_3] Changes	2.5% →	9% CO_2	% Change	50 mM →	13.5 mM HCO_3	% Change
Blank	99.0 ± 1.0	89.5 ± 1.3**	−9.5 ± 0.6	105.0 ± 1.0	94.4 ± 0.9**	−10.6 ± 1.5
d-Tubocurarine	58.7 ± 2.7	30.5 ± 3.1**	−28.2 ± 1.6	44.9 ± 5.5	12.8 ± 3.3**	−32.0 ± 2.9
Vecuronium	68.5 ± 3.1	45.5 ± 3.1**	−23.0 ± 1.4†	87.7 ± 1.9	50.9 ± 3.9**	−36.8 ± 3.2†
Metocurine	50.3 ± 6.3	51.5 ± 5.6	+1.2 ± 1.1	40.3 ± 4.8	43.3 ± 3.9*	+2.9 ± 1.3
Pancuronium	30.7 ± 4.4	38.9 ± 4.2**	+8.3 ± 1.5	46.8 ± 6.4	58.3 ± 3.9**	+11.5 ± 3.0

Each value is expressed as per cent of control twitch tension in the 5% CO_2 and 25 mM HCO_3 solution (mean ± SEM, n = 8). Each value in % change column represents amount of change in twitch after PCO_2 or [HCO_3] manipulation.

* and ** indicate significant differences from the value prior to each manipulation of PCO_2 or [HCO_3] at $P < 0.05$ and <0.01 levels, respectively (paired *t* test).

There is no significant difference in the amount of change in twitch with or without each neuromuscular blocking agent between that after P_{CO_2} manipulation and that after HCO_3 replacement, with the exception of vecuronium, as indicated by † (analysis of variance).

lengths of time of exposure to each HCO_3 solution presumably caused the above difference.

After a partial depression of twitch by the neuromuscular blocking agents, the tracings with [HCO_3] manipulations were different from those with CO_2 manipulations (fig. 2). In the former, there was an overshoot of twitch before stabilization. This was not apparent in the tracings without the blocking agents and presumably was due to the transient removal of the blocking agents from the neuromuscular junction during replacement of the bathing fluid. After the overshoot, a stabilization of twitch followed in the same way as it did with the manipulation of P_{CO_2}. Decreasing *p*H by the manipulation of P_{CO_2} or [HCO_3] was accompanied by a significant decrease in twitch after the treatment with dTc ($P < 0.01$) or vecuronium ($P < 0.01$), whereas it was accompanied by a significant increase in twitch after treatment with metocurine (not significant when induced by CO_2; $P < 0.05$ when induced by [HCO_3]) or pancuronium ($P < 0.01$). Conversely, increasing *p*H accompanied a significant increase in twitch after the treatment with dTc ($P < 0.01$) or vecuronium ($P < 0.01$), whereas it accompanied a significant decrease in twitch after the treatment with metocurine ($P < 0.01$) or pancuronium ($P < 0.01$). Although not shown in tables 3 and 4, the amounts of change in twitch with each neuromuscular blocking agent were significantly different from those without the blocking agents ($P < 0.05$, analysis of variance and Bonferroni t test). There was no significant difference in the amounts of change in block by each blocking agent between those after P_{CO_2}-induced *p*H changes and those after [HCO_3]-induced similar *p*H changes, with the exception of vecuronium and metocurine (indicated by † in Table 3 and 4).

Discussion

The study demonstrates that acid–base changes, whether induced by P_{CO_2} manipulations or by [HCO_3] manipulations, cause similar changes in indirectly elicited twitch tension with and without prior administration of nondepolarizing neuromuscular blocking agents. The force of the evoked twitch was greater at a higher *p*H

TABLE 4. Effects of Increasing *p*H Caused by Decreasing CO_2 or Increasing [HCO_3] on Twitch Tension with and without *d*-Tc, Vecuronium, Metocurine, and Pancuronium

	Values and Amounts of Change in Twitch Tension					
CO_2 or [HCO_3] Changes	9% →	2.5% CO_2	% Change	13.5 mM →	50 mM HCO_3	% Change
Blank	89.6 ± 1.8	95.2 ± 1.9**	+5.6 ± 0.9	79.4 ± 0.7	87.2 ± 1.1**	+7.9 ± 0.6
d-Tubocurarine	39.2 ± 5.5	52.0 ± 4.6**	+12.8 ± 2.2	7.2 ± 2.4	20.9 ± 2.3**	+13.6 ± 1.8
Vecuronium	27.2 ± 6.2	52.5 ± 5.8**	+25.3 ± 1.7	31.2 ± 6.4	56.2 ± 3.8**	+25.0 ± 3.0
Metocurine	48.4 ± 4.6	44.2 ± 4.8**	−4.2 ± 0.6†	43.3 ± 1.0	35.2 ± 1.6**	−8.0 ± 1.1†
Pancuronium	29.7 ± 4.8	18.7 ± 3.8**	−11.0 ± 1.2	41.0 ± 5.6	23.5 ± 5.0**	−17.5 ± 2.0

Each value is expressed as % of control twitch tension in the 5% CO_2 and 25 mM HCO_3 solution (mean ± SEM, n = 8). Each value in % change column represents amount of change in twitch after P_{CO_2} or [HCO_3] manipulation.

** Indicates a significant difference from the value prior to each manipulation of P_{CO_2} or [HCO_3] at $P < 0.01$ level (paired *t* test).

There is no significant difference in the amount of change in twitch with or without each neuromuscular blocking agent between that after P_{CO_2} manipulation and that after HCO_3 replacement with the exception of metocurine indicated by † (analysis of variance).

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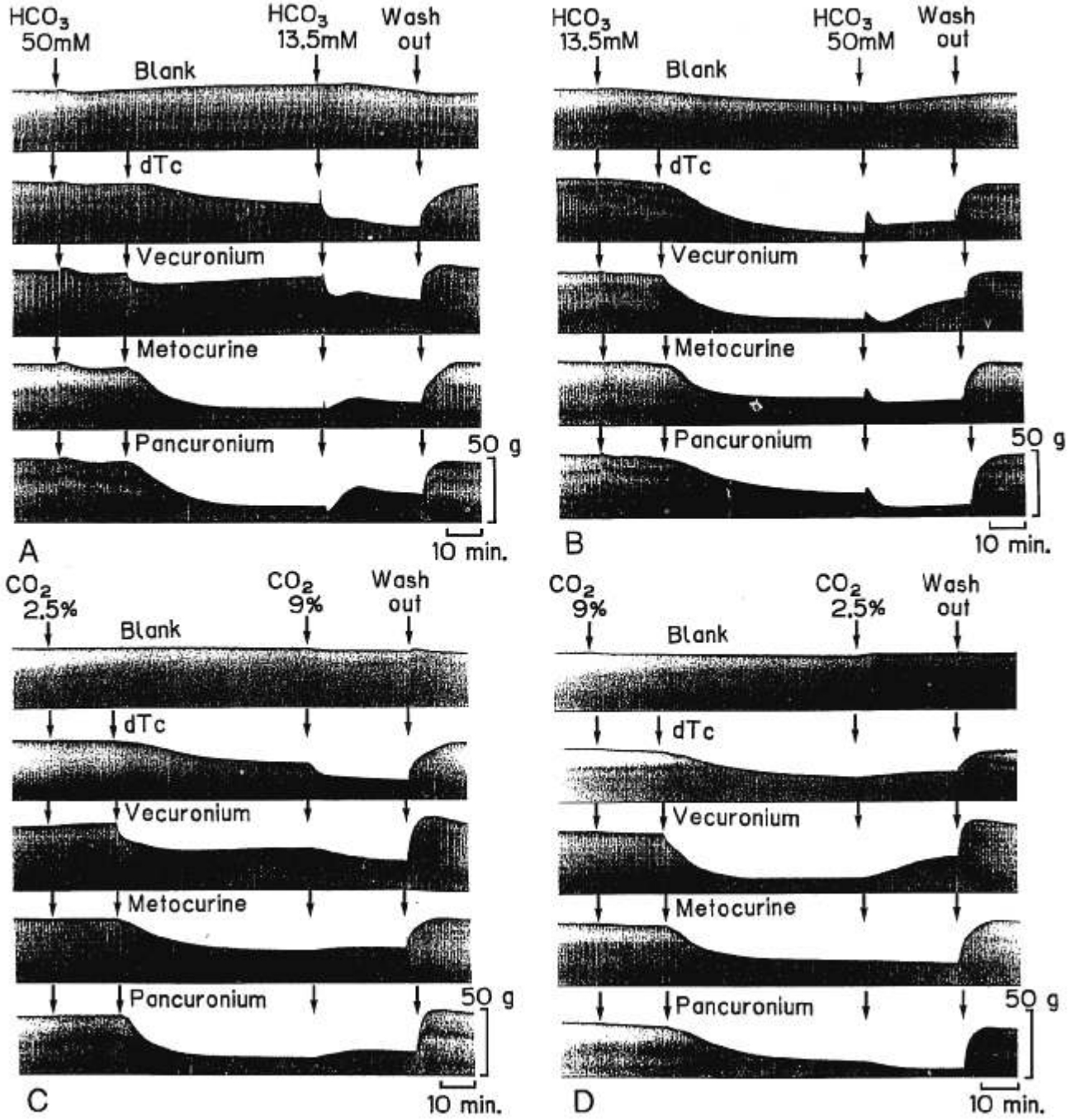

FIG. 2. Effects of *p*H changes caused by P_{CO_2} or [HCO_3] with and without dTc, vecuronium, metocurine and pancuronium. In the absence of neuromuscular blocking agents, decreasing *p*H either by decreasing [HCO_3] or increasing P_{CO_2} decreased twitch, and increasing *p*H either by increasing [HCO_3] or decreasing P_{CO_2} increased it. After pretreatment with *d*Tc or vecuronium, decreasing *p*H decreased twitch, whereas it increased twitch after pretreatment with metocurine or pancuronium (*A, C*). Increasing *p*H increased twitch pretreated with dTc or vecuronium, whereas it decreased twitch pretreated with metocurine or pancuronium, (*B, D*).

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than at a lower *p*H, whether induced by changes in P_{CO_2} or in [HCO_3]. Previous studies[12] reported substantial evidence to indicate that intracellular rather than extracellular *p*H change is responsible for the results. Our finding that CO_2 changed twitch rapidly, whereas [HCO_3] did so slowly, is consistent with those of Creese[13] and also reflects the intracellular site of action of *p*H changes.

In contrast, the change in neuromuscular block was the same no matter how *p*H was modified, and was different according to the blocking agents used. Neuromuscular block by either dTc or vecuronium was augmented by decreasing *p*H and reduced by increasing *p*H. Neuromuscular block by either metocurine or pancuronium was reduced by decreasing *p*H and augmented by increasing *p*H. The amount of change in block caused by the manipulation of P_{CO_2} was similar to that caused by the manipulation of [HCO_3]. These findings suggest that neither P_{CO_2} nor [HCO_3] has a specific action, but rather that changes in *p*H, presumably extracellular *p*H changes, are responsible for the results with the neuromuscular blocking agents.

The different results in the dTc and vecuronium group and the metocurine and pancuronium group suggest that the above observations may be attributed to the difference in chemical structure of these compounds, that is, the monoquaternary nature of dTc and vecuronium as opposed to the bisquaternary structures of metocurine and pancuronium.[9] At a lower *p*H, the charge density of a tertiary ammonium group in the monoquaternary compounds would be increased by combining with a hydrogen ion, and pseudo-bisquaternary compounds would be formed. These changes in the degree of ionization of drugs and the resulting increase in the affinity to anionic acetylcholine receptors may account for the *p*H-induced changes in the potencies of the monoquaternary dTc and vecuronium group. Regarding the opposite changes in the potencies of the bisquaternary metocurine and pancuronium group, *p*H-induced changes in the ionization of anionic acetylcholine receptors may be responsible for the results.

Alterations in extracellular [Ca^{2+}] accompany acid–base changes *in vivo*. The competition between hydrogen ion and Ca^{2+} for protein-binding sites is responsible for these changes in [Ca^{2+}]. Even in the protein-free physiologic solution *in vitro*, Ca-complexes have been reported to be formed with ligands such as phosphate, citrate, and HCO_3. It follows that alterations in [Ca^{2+}] accompany the [HCO_3] changes in the modified Krebs' solution.[14,15] Similarly, [Mg^{2+}] varies during [HCO_3]-induced acid–base changes. In contrast, alterations in P_{CO_2} did not change [Ca^{2+}] or [Mg^{2+}] *in vitro*. This study was designed to compare the neuromuscular effects of respiratory and metabolic *p*H changes *in vitro* and to eliminate other variables. To separate the neuromuscular effects of changes in extracellular [Ca^{2+}] and [Mg^{2+}][11,§] from those of *p*H changes, the experiments with [HCO_3] were done with replacement of Ca and Mg. When [Ca^{2+}] and [Mg^{2+}] were kept constant, the change in twitch was the same, whether due to [HCO_3] or P_{CO_2} manipulations.

Our results are consistent with those of Kalow,[3] who demonstrated in the frog rectus muscle that dTc inhibited muscle contraction more in acid than in alkaline solution, whereas metocurine inhibited less in acid solution. Funk *et al.*[16] showed in the rat hemidiaphragm only minimal vecuronium block antagonism when the *p*H was raised from 7.4 to 7.68 with sodium carbonate infusion, and significant potentiation under acidic conditions (*p*H 7.05) with HCl infusion. This resulted presumably because these investigators studied *p*H increase of a lesser degree compared to that in our study (0.28 *vs.* 0.5 *p*H units, respectively). Our results are inconsistent with those of Crul-Sluijter,[7] who reported, using the same preparation as our study, that either HCl or 5,5-dimethyl-2,4-oxazolidinedione (DMO) infusion caused an insignificant potentiation in pancuronium block. They changed the *p*H from 7.39 to 7.05 by a 10-min infusion of HCl or DMO, whereas we changed the *p*H from 7.72 to 7.19 by replacement of [HCO_3] solutions with [Ca^{2+}] and [Mg^{2+}] values kept constant. Although there are several differences in experimental design between Crul-Sluijter's study and ours, we have no clear answer to explain this opposite change.

In conclusion, the alteration of *p*H *in vitro* caused similar changes in indirectly elicited twitch tension, whether the alteration was induced by changes in [HCO_3] or in P_{CO_2}. Decreasing *p*H caused a decrease in twitch. Increasing *p*H increased twitch. A decrease in *p*H potentiated dTc and vecuronium block, whereas it antagonized metocurine and pancuronium block. An increase in *p*H antagonized dTc and vecuronium block, whereas it potentiated metocurine and pancuronium block. These findings suggest that neither P_{CO_2} nor [HCO_3] has a specific action, but that changes in *p*H may be responsible for the results. In addition, monoquaternary neuromuscular blocking (dTc and vecuronium) agents respond to acid–base changes differently than do bisquaternary neuromuscular blocking (metocurine and pancuronium) agents.

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