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Editorial Views

The Effect of Acid–Base Balance on Neostigmine Antagonism of d-*Tubocurarine-induced Neuromuscular Blockade*

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d-Tubocurarine (*d*Tc) was infused intravenously into 35 cats anesthetized with chloralose and urethane at a constant continuous rate to produce and maintain 90 per cent depression of twitch height of the anterior tibial muscle following supramaximal stimulation of the peroneal nerve. The mean infusion rates that produced 90 per cent depression were not significantly altered by respiratory acid–base changes. Metabolic alkalosis decreased (32.5 per cent) and metabolic acidosis increased (27.7 per cent) the required infusion rate of *d*Tc. When *p*H and Pa_{CO_2} were maintained at 7.37 and 38 torr, respectively, the addition of a bolus of neostigmine, 10.5 μg/kg, intravenously, to the continuing infusion of *d*Tc produced 50 per cent antagonism of the *d*Tc-depressed twitch. Respiratory alkalosis and metabolic acidosis did not alter the dose of neostigmine needed to produce 50 per cent antagonism. However, during respiratory acidosis (*p*H 7.13, Pa_{CO_2} 66 torr) and metabolic alkalosis (*p*H 7.59, Pa_{CO_2} 36 torr) 20.0 and 18.0 μg/kg neostigmine, respectively, were needed to produce 50 per cent antagonism. Still larger doses of neostigmine (75 μg/kg) could not completely antagonize the block unless *p*H and Pa_{CO_2} were returned to 7.30–7.50 and 35–45 torr, respectively. It is concluded that respiratory acidosis and metabolic alkalosis limit and oppose antagonism of *d*Tc by neostigmine. (Key words: Neuromuscular relaxants, *d*-tubocurarine; Acid–base equilibrium, neuromuscular relaxants; Antagonists, neuromuscular relaxants, neostigmine.)

"NEOSTIGMINE-RESISTANT CURARIZATION" has been attributed, in part, to metabolic and respiratory acidosis.[1–3] We tested this hypothesis by measuring the alterations in the ability of neostigmine to antagonize *d*-tubocurarine (*d*Tc)-induced neuromuscular blockade during respiratory and metabolic acidosis and alkalosis.

Methods

Thirty-five cats, 2 to 4.5 kg in weight, were anesthetized with chloralose, 80 mg/kg, and urethane, 250 mg/kg, intraperitoneally. After a tracheostomy was performed, ventilation was controlled via a Harvard volume ventilator. The tendon of the anterior tibialis muscle was freed, sectioned near its point of attachment, and connected to a Grass FT. 03 force-displacement transducer. The sciatic nerve was sectioned. The distal peroneal

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TABLE 1. Numbers of Cats Studied, Acid–Base Values, and Infusion Rates of *d*Tc Required for 90 Per Cent Depression of Twitch Height

	Number of Cats	*p*H	Pa_{CO_2} (torr)	Infusion Rate (μg/kg/min)
Control	5	7.37 ± 0.01	38.0 ± 0.9	8.6 ± 0.3
Respiratory alkalosis	5	7.53 ± 0.02	17.4 ± 0.4	9.3 ± 0.4
Respiratory acidosis	7	7.13 ± 0.01	66.1 ± 0.8	8.5 ± 0.4
Metabolic alkalosis	9	7.59 ± 0.01	36.3 ± 0.7	5.8 ± 0.3
Metabolic acidosis	6	7.01 ± 0.02	37.7 ± 0.6	11.0 ± 0.6

nerve was isolated and supramaximal stimuli (2 to 10 volts) of 0.3 msec duration and 0.1 Hz were applied from a Grass stimulator (Model S4G) through shielded platinum electrodes to stimulate the muscle indirectly. The resulting force of muscle contraction was continuously recorded on a polygraph as discrete twitches in such a manner that twitch height was proportional to isometric contractile force.

A bolus intravenous injection of *d*Tc, 0.2 mg/kg, then was given, after which *d*Tc, 100 μg/ml, was infused from a Harvard pump at a rate that produced a constant 90 per cent depression of twitch height. The rate of infusion necessary decreased progressively for the next 15–45 minutes, after which it remained constant, as determined by at least 15 minutes of observation. Further details of this constant-infusion technique have been described.[4] While the *d*Tc infusion was continued at this rate, neostigmine was administered as an intravenous bolus. The resultant maximum antagonism of twitch depression was recorded and is presented as a percentage of the pre-existing 90 per cent depression (*e.g.*, a peak rise to 40 per cent of the pre-*d*Tc twitch would be calculated as (40−10)100/90, or 33 per cent antagonism). In addition, we measured times from neostigmine administration to peak effect (onset time) and to 70, 50, and 30 per cent return to the *d*Tc-depressed twitch height (duration of action). Subsequent doses of neostigmine were given after the twitch had returned to 90 per cent depression for at least 15 minutes.

Acid–base values and arterial carbon dioxide tensions (Pa_{CO_2}) were stable for at least 30 minutes before infusion of *d*Tc was initiated. Arterial blood–gas values were obtained immediately before and approximately 15–20 minutes and 50–60 minutes after neostigmine administration. Hypocarbia was produced by increasing the respiratory rate of the Harvard volume ventilator. Hypercarbia was produced by adding carbon dioxide to the inspired gases. During hypocarbia and hypercarbia base excess was maintained between minus 10 and zero mEq/l. Metabolic alkalosis and metabolic acidosis were produced by constant infusion of 5 M sodium bicarbonate (six cats), sodium carbonate (three cats), or .1 M hydrochloric acid (six cats).

Serum sodium, potassium, calcium, and chloride levels were determined immediately before and two and five hours after the acid–base changes were instituted.

Analysis of variance was used for part of the statistical analyses.[5] Linear regression analysis and unpaired t-test were carried out for the remaining results.[5]

Results

Mean Pa_{CO_2} and *p*H for each group of cats are summarized in table 1. Although the mean infusion rate of *d*Tc required to maintain a constant 90 per cent depression of twitch height was less during respiratory acidosis than during respiratory alkalosis, the difference was not significant (table 1). In contrast, the infusion rate of *d*Tc was significantly less during metabolic alkalosis and significantly more during metabolic acidosis than the control infusion rate ($P < 0.01$) (table 1).

Neither respiratory alkalosis nor metabolic acidosis altered the dose of neostigmine needed to antagonize *d*Tc-induced neuromuscular blockade (figs. 1 and 2). However, respiratory acidosis (excluding the 50 and

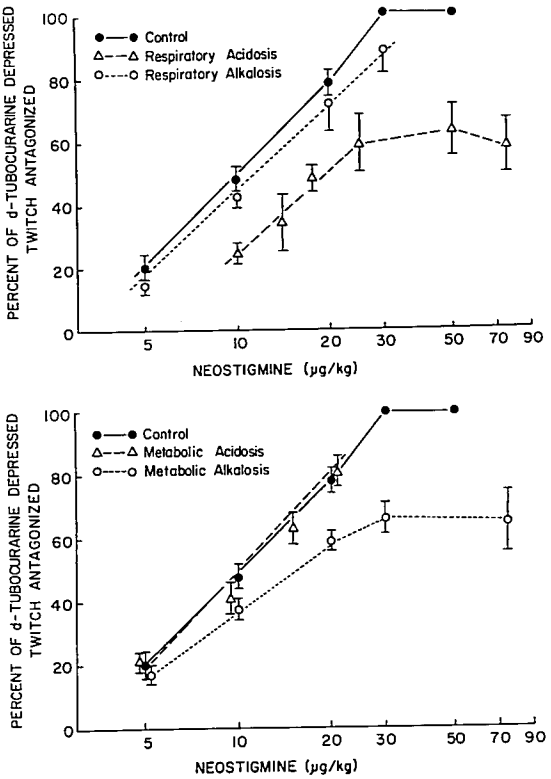

FIG. 1. (*above*) and 2 (*below*). Correlation by dose of neostigmine and percentage of *d*Tc-depressed twitch antagonized. Each symbol represents the mean ± 1 SE. The number of cats studied at each dose can be obtained from table 2.

75 μg/kg doses) shifted the dose–response curve of neostigmine antagonism to the right significantly in a parallel manner (fig. 1) ($P < 0.01$). For example, the dose of neostigmine required for 50 per cent antagonism of the *d*Tc-depressed twitch was 10.5 μg/kg during normal acid–base status and 20 μg/kg during respiratory acidosis (fig. 1). Additionally the large doses of neostigmine (50 and 75 μg/kg) were able to antagonize only about 60 per cent of the *d*Tc-depressed twitch (fig. 1). The *d*Tc-induced blockade was entirely antagonized only when Pa_{CO_2} was returned to normal. Similarly, more neostigmine was required to antagonize the *d*Tc-depressed twitch during metabolic alkalosis produced by infusion of sodium bicarbonate (fig. 2). Although not displayed in figure 2, metabolic alkalosis induced by sodium carbonate (*p*H 7.59, Pa_{CO_2} 37 torr) also limited and opposed antagonism of *d*Tc by neostigmine. Neostigmine, 10, 20, 30, and 75 μg/kg, caused 23 ± 5.2, 42 ± 7.9, 28 ± 0.5 and 29 ± 5.5 per cent antagonism of

TABLE 2. Onset and Duration (Mean ± SE) of Antagonism of *d*Tc-depressed Twitch Height by Neostigmine

Dose of Neostigmine (μg/kg)	Number of Cats	Acid–Base Status	Onset Time* (Min)	Duration (Min)†		
				70 Per Cent	50 Per Cent	30 Per Cent
5	5	Control	6.0 ± 0.9	14.9 ± 1.9	19.1 ± 2.5	25.2 ± 5.7
10	5		7.1 ± 0.7	14.9 ± 1.9	24.6 ± 2.7	35.3 ± 5.1
20	5		7.7 ± 0.9	23.4 ± 2.9	31.5 ± 3.9	44.3 ± 6.1
30	2		4.5 ± 1.1	37.1 ± 11.1	51.9 ± 10.7	74.7 ± 13.9
50	2		4.9 ± 1.6	58.9 ± 14.1	78.5 ± 19.5	108.0 ± 17.2
5	4	Respiratory alkalosis	6.7 ± 1.2	21.8 ± 4.0‡	30.2 ± 3.3‡	36.4 ± 5.5
10	5		7.5 ± 0.9	22.0 ± 2.2‡	30.7 ± 4.6	37.8 ± 3.7
20	4		7.1 ± 0.6	23.3 ± 2.8	35.7 ± 5.8	46.7 ± 5.8
30	5		6.4 ± 0.4	18.5 ± 2.5	35.3 ± 4.2	56.2 ± 8.3
10	7	Respiratory Acidosis	8.1 ± 0.9	23.8 ± 1.6	27.0 ± 3.8	35.2 ± 2.8
14	4		9.4 ± 1.5	28.2 ± 0.9	30.4 ± 5.1	47.3 ± 2.9
17.5	4		11.8 ± 0.2	25.6 ± 0.8	38.0 ± 3.7	57.9 ± 6.0
25	4		11.3 ± 1.9	37.8 ± 5.6	44.8 ± 7.3	60.4 ± 10.2
50	4		8.3 ± 0.9	25.9 ± 2.8‡	40.2 ± 3.5‡	68.6 ± 2.8‡
75	5		8.5 ± 1.3	48.5 ± 7.3	69.0 ± 10.1	82.9 ± 13.0
5	4	Metabolic alkalosis	8.2 ± 1.0	18.1 ± 1.0	21.2 ± 3.2	28.8 ± 1.2
10	5		8.9 ± 0.6	21.2 ± 1.8	30.2 ± 3.7	32.5 ± 4.8
20	6		7.4 ± 0.6	26.0 ± 0.7	40.6 ± 3.6	52.2 ± 3.2
30	5		5.9 ± 0.2	18.6 ± 2.8	27.8 ± 4.6	40.2 ± 6.6
75	5		4.5 ± 0.2	20.9 ± 1.0	45.7 ± 1.8	—§
5	5	Metabolic acidosis	10.6 ± 1.3‡	21.7 ± 3.2	25.4 ± 3.3	31.0 ± 5.4
10	7		10.5 ± 1.1‡	25.1 ± 4.3	35.3 ± 7.5	42.4 ± 7.2
15	6		8.5 ± 0.9	27.2 ± 3.6	36.6 ± 4.7	47.7 ± 6.2
20	6		12.1 ± 2.2‡	33.6 ± 4.8	45.9 ± 5.9	49.0 ± 4.5

* Time from neostigmine administration to peak antagonism of the *d*Tc-depressed twitch height.

† Time from neostigmine administration to 70, 50, and 30 per cent return to the *d*Tc-depressed twitch height.

‡ Significantly different from those values obtained during control acid–base status ($P < 0.05$).

§ Not determined.

the *d*Tc-depressed twitch, which was less antagonism than the same doses of neostigmine produced during the control state ($P < 0.01$). Pa_{O_2} was never lower than 80 torr. As with respiratory acidosis, the *d*Tc-depressed twitch was not completely antagonized by neostigmine during metabolic alkalosis unless arterial-blood *p*H was first returned to between 7.35 and 7.45 by infusion of hydrochloric acid. Times to onset of action of 5, 10, and 20 μg/kg doses of neostigmine were prolonged during metabolic acidosis ($P < 0.05$) (table 2).

The onset times for neostigmine during metabolic and respiratory alkalosis and respiratory acidosis were not different from those observed during the control state. Although the onset times appeared to be longer with the 17.5 and 25 μg/kg doses of neostigmine during respiratory acidosis, statistical analysis was not performed because these doses were not given during the control acid–base state. Except in two instances, duration of action of neostigmine was not affected by changes in acid–base status. During respiratory alkalosis, the times from administration of neostigmine, 5 μg/kg, to 70 and 50 per cent return to the *d*Tc-depressed twitch height (duration of action) were significantly longer than in controls ($P < 0.05$) (table 2). In contrast, times to equal recoveries were shorter following neostigmine,

50 μg/kg, during respiratory acidosis than during the control acid–base state (table 2).

No change in serum sodium, chloride, or calcium was observed during respiratory acidosis and alkalosis. During metabolic acidosis, serum sodium decreased from 151 ± 1 to 138 ± 1 mEq/l, serum potassium increased from 4.30 ± 0.2 to 5.1 ± 0.1 mEq/l, and serum calcium decreased from 7.7 ± 0.1 to 6.8 ± 0.3 mEq/l ($P < 0.01$). During metabolic alkalosis, serum sodium increased from 152 ± 1 to 167 ± 2 mEq/l, while serum potassium and calcium decreased from 4.6 ± 0.2 and 7.6 ± 0.2 mEq/l to 3.5 ± 0.2 and 6.2 ± 0.4 mEq/l, respectively ($P < 0.01$).

Discussion

Our results partially conflict with those of other investigators who suggest that metabolic or respiratory acidosis produces neostigmine-resistant curarization.[1–3] We found neostigmine-resistant curarization with respiratory acidosis and metabolic *alkalosis*. Furthermore, during metabolic alkalosis neostigmine did not antagonize the block despite significantly less *d*Tc being infused to maintain 90 per cent depression of twitch height (table 1). Complete antagonism was accomplished only when *p*H was returned to between 7.3 and 7.5 by decreasing the inspired carbon dioxide concentration, by augmenting ventilation during respiratory acidosis, or by infusing hydrochloric acid during metabolic alkalosis.

The ability of neostigmine to antagonize *d*Tc does not depend on extracellular *p*H. For example, metabolic acidosis (*p*H 7.01) had no effect, but respiratory acidosis (*p*H 7.13) limited and opposed antagonism of *d*Tc. In contrast to the lack of correlation with extracellular *p*H, changes in intracellular *p*H may correlate with the ability of neostigmine to antagonize *d*Tc. Infusion of hydrochloric acid will not change and hyperventilation will increase intracellular *p*H.[6] Neither of these changes affects neostigmine antagonism of *d*Tc. On the other hand, infusion of sodium bicarbonate will slightly decrease and hypoventilation will markedly decrease intracellular *p*H.[7–10] Both of these conditions oppose antagonism of *d*Tc by neostigmine. Perhaps any condition that produces an intracellular acidosis will oppose the ability of neostigmine to antagonize *d*Tc.

More likely the reduced *d*Tc requirement and "neostigmine-resistant curarization" during metabolic alkalosis can be explained by changes in serum electrolytes. For example, although not changed by respiratory acidosis or alkalosis, serum potassium decreased during metabolic alkalosis. A decrease in extracellular potassium may increase the resting transmembrane potential, which would resist the action of acetylcholine and, therefore, neostigmine.[11] This might also reduce the amount of *d*Tc required for neuromuscular blockade. Consistent with this hypothesis, metabolic acidosis increased both serum potassium and the rate of *d*Tc infusion necessary for 90 per cent reduction in twitch height.

How might we explain the difference between the conclusions of Brooks and Feldman[1] and our results? Brooks and Feldman based their conclusions on two patients whose neuromuscular blockades following 40 and 85 mg *d*Tc could not be antagonized by 3.75 and 2.5 mg neostigmine, respectively. Although metabolic acidosis may have been a contributing factor, the doses of *d*Tc may have produced neuromuscular blockades too intense for neostigmine to antagonize.[12] Another factor may be the manner in which metabolic acidosis was induced. Clinically, metabolic acidosis usually is induced by cellular hypoxia secondary to hypoxemia and/or hypoperfusion, which is more likely to lead to intracellular acidosis than is metabolic acidosis produced by infusion of hydrochloric acid.[13] Since Pa_{O_2} was always more than 80 torr in our studies, hypoxia at the neuromuscular junction was unlikely. In any event, metabolic acidosis, *per se*, does not interfere with antagonism of *d*Tc by neostigmine.

Although we are the first to study the effect of acid–base changes on neostigmine antagonism of *d*Tc, several investigators have studied the effect of these changes on *d*Tc-induced blockade itself. The results of these studies indicate that respiratory acidosis

either augments or does not alter, while respiratory alkalosis either antagonizes or does not alter, *d*Tc-induced blockade.[14,15] However, there is little agreement concerning the influence of metabolic acid–base changes. This lack of agreement may be due to differences in experimental protocol. In contrast to our study, Gamstorp and Vinnars[16] found that metabolic alkalosis antagonized and metabolic acidosis augmented *d*Tc-induced blockade. In their studies Pa_{CO_2} was lower during alkalosis and significantly higher during acidosis than during the control period; the changes in *p*H were, therefore, both respiratory and metabolic in origin. Was it the elevated Pa_{CO_2} rather than metabolic acidosis that augmented *d*Tc-induced blockade? Katz *et al.* concluded that metabolic alkalosis slightly antagonized *d*Tc-induced blockade when the *p*H increased from 0.2 to 0.48 units and Pa_{CO_2} decreased a mean of 7 torr (range 2 to 12 torr).[17] Is this metabolic or respiratory alkalosis? In our studies ventilation was controlled to maintain Pa_{CO_2} at control levels during the metabolic studies (table 1).

Consistent with our results, Payne[18] found that sodium bicarbonate-induced metabolic alkalosis enhanced *d*Tc-induced blockade. Yet, using sodium carbonate, Katz[17] and Gamstorp and Vinnars[16] found that metabolic alkalosis antagonized *d*Tc-induced blockade. We believe that the difference in alkali infused is of little consequence, since both enhanced *d*Tc-induced blockade in our studies. We produced metabolic alkalosis by a constant infusion with *p*H stable for at least 30 minutes before administration of *d*Tc. Other investigators produced metabolic alkalosis by a single injection of alkali, which probably means that *p*H was unstable during *d*Tc administration.[16,17]

In contrast to other studies, we measured the effect of acid–base changes on neuromuscular blockade produced by constant infusion of *d*Tc rather than by a single bolus injection. We believe the constant-infusion technique eliminates several extraneous factors that may influence the blockade. With constant infusion and blockade, distribution of blood flow and uptake of *d*Tc by tissue depots cannot influence the effective dose at the neuromuscular junction. In contrast, variations in tissue and plasma protein binding induced by changes in *p*H, bicarbonate, or other ions would influence the amount of *d*Tc reaching the neuromuscular junction following bolus injection. An increase in binding would decrease the effectiveness of a given dose of *d*Tc. Similarly, if blood flow to skeletal muscle were decreased (as occurs during metabolic alkalosis),[19] then less of the injected *d*Tc would reach the neuromuscular junction. This would falsely suggest that metabolic alkalosis antagonizes the *d*Tc-induced blockade.

The increased potency of *d*Tc during metabolic alkalosis and reduced effectiveness of neostigmine during both metabolic alkalosis and respiratory acidosis may make *d*Tc administration hazardous during these altered acid–base states. For example, if a patient in the recovery room hypoventilates from residual anesthesia, attempts to antagonize residual *d*Tc-induced paralysis may be unsuccessful; in fact, hypoventilation may be augmented by the residual paralysis. Administration of narcotics to relieve pain may add to the likelihood of this sequence of events.

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CNS

NEUROMUSCULAR TRANSMISSION IN DIABETICS Neuromuscular transmission has been measured electromyographically in 30 diabetic patients having one or more signs of peripheral neuropathy at clinical or electromyographic examination, together with 20 normal subjects. The median nerve was stimulated supramaximally with a bipolar stimulator strapped at the wrist. Trains of 20–24 0.1-msec stimuli were delivered at frequencies of 2–500/sec. Peak-to-peak action potential amplitude of the twentieth response as percentage of the first response of each train was plotted against pulse frequency. Rapid early fatigability was evident in most patients, and stimulus frequencies of 50·sec^{-1} and 100·sec^{-1} produced decrements in amplitudes of evoked electrical responses in diabetic patients, but facilitation in normal subjects. The decrement of the muscle electrical response was qualitatively similar to that seen in myasthenia gravis. Median-nerve sensory action potentials were recorded antidromically in five normal subjects and ten diabetic patients. Conduction velocity and the distal latency of the median nerve were within normal limits in all patients. Failure of neuromuscular transmission could, therefore, constitute the sole evidence of incipient neuronal failure. Failure of the neuromuscular apparatus could produce the symptoms of weakness and fatigability that are common in diabetes. The authors suggest several possible etiologic mechanisms. (*Miglietta, O.: Neuromuscular Junction Defect in Diabetics. Diabetes 22:719–723, 1973.*)