The Influence of Mild Hypothermia on the Pharmacokinetics and Time Course of Action of Neostigmine in Anesthetized Volunteers

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Background: The pharmacokinetics, maximum effect, and time course of action of neostigmine were studied in seven human volunteers.

Methods: Each volunteer was studied twice, during both normothermia and hypothermia. Anesthesia was induced with 30 μg/kg alfentanil and 3 mg/kg propofol, and was maintained with 60–70% nitrous oxide and 0.7–0.9% isoflurane. The mechanical response of the adductor pollicis to train-of-four stimulation of the ulnar nerve was recorded, and central body temperature maintained stable at either less than 34.5°C or greater than 36.5°C by surface cooling or warming. Before neostigmine administration, a stable 5% twitch height was obtained by an infusion of vecuronium, and the infusion rate remained unchanged thereafter. Neostigmine, 70 μg/kg, was then infused over 2 min, and blood samples for estimation of neostigmine concentrations were collected at intervals for 240 min.

Results: With hypothermia, the central volume of distribution of neostigmine decreased by 38%, and onset time of maximum effect increased (4.6 *vs.* 5.6 min). Hypothermia did not change the clearance (696 ml/min), maximum effect, or duration of action of neostigmine.

Conclusions: The efficacy of neostigmine as an antagonist of vecuronium-induced neuromuscular block is not altered by mild hypothermia.

IN a previous study, we demonstrated that a reduction in central body temperature of 2°C more than doubled the duration of action of vecuronium.[1] This prolonged duration of muscle relaxation may be an explanation for why hypothermia is quoted as a potential cause of inadequate reversal of neuromuscular block.[2] However, hypothermia may also affect the pharmacology of neostigmine, and a decreased efficacy of neostigmine with hypothermia could contribute to inadequate reversal observed in the postoperative period.[3,4] We hypothesize that mild hypothermia might diminish the efficacy of neostigmine

and alter its pharmacokinetics. Thus, we investigated the effect of hypothermia on the efficacy and pharmacokinetics of neostigmine, in isolation from any effect of temperature on the duration of action of vecuronium. To do this, we performed the study in human volunteers during a constant infusion of vecuronium, using previously described methodology.[5]

Materials and Methods

The institution's Committee on Human Research (University of California, San Francisco, California) approved the study, and informed consent was obtained from all participants. Seven unpremedicated, healthy, nonobese volunteers not taking chronic medication were included. Each volunteer was studied twice, 1 week apart, once at normothermia and once at hypothermia. The assignment to normothermia or hypothermia on the first study day was done on a random basis. On the second study day, experimental conditions were replicated, with the exception of the central body temperature. Standard vital signs monitoring was performed according to the guidelines of the American Society of Anesthesiologists and clinical standards practiced at the Medical Center of the University of California, San Francisco.

An infusion of lactated Ringer's solution and all study medications were administered *via* an 18-gauge cannula inserted into a vein in the subject's right arm. Anesthesia was induced with 30 μg/kg alfentanil and 3 mg/kg propofol. Tracheal intubation was then performed without the use of neuromuscular blocking agents. Anesthesia was maintained with 60–70% nitrous oxide in oxygen and 0.7–0.9% isoflurane end-tidal concentration. Mechanical ventilation was adjusted to maintain end-tidal carbon dioxide at 30–35 mmHg. Following induction of anesthesia, a 16-gauge intravenous cannula was inserted in the subject's left arm for obtaining blood samples.

Supramaximal stimuli (0.2 ms duration) in a train-of-four (TOF) sequence at 2 Hz were applied every 12 s *via* surface electrodes to the ulnar nerve at the left wrist (Digistim II; Neuro Technology Inc., Houston, TX). The resulting evoked mechanical responses of the adductor pollicis (preload 200–300 g) were measured with a calibrated force transducer (Myotrace; Life-Tech Inc., Houston, TX) and were amplified (DC Signal Conditioner; Gould Electronics, Valley View, OH). Twitch tension of the first TOF response (T1) and the ratio of the fourth to the first response (TOF ratio) were digitized, displayed,
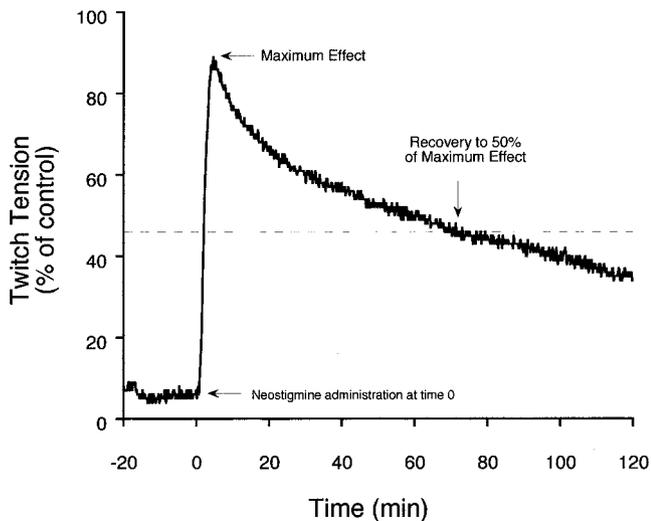
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Fig. 1. Neuromuscular response data from one subject. Neostigmine, 70 μg/kg, was administered at time zero. Control twitch tension was obtained after the volunteer had attained and stabilized at the desired study temperature and before vecuronium administration.

and recorded on a Macintosh IIci computer (LabView; National Instruments, Austin, TX).

Central body temperature was measured with a thermocouple in the distal esophagus. For normothermia, the central temperature was greater than 36.5°C, and for hypothermia, the central temperature was less than 34.5°C. Normothermia was achieved by forced-air warming (Bair Hugger; Augustine Medical, Minneapolis, MN), and hypothermia was achieved by surface cooling with wet towels and a fan. Once the subject's temperature was in the target range and had stabilized, that temperature was maintained throughout the study within ±0.1°C.

When the subject's temperature was in the hypothermic or normothermic range and had been stable at ±0.1°C and the adductor pollicis twitch tension had been stable for 15 min, the T1 response of TOF was used as the control twitch to which all subsequent T1 responses were compared. Vecuronium was then administered as a bolus of 0.03 mg/kg, and an infusion was started and adjusted to achieve a T1 response of 5 ± 1%. The vecuronium infusion was subsequently maintained at this rate for the remainder of the study. When the T1 response had been stable at 5 ± 1% for at least 10 min, 70 μg/kg neostigmine (preceded by 14 μg/kg glycopyrrolate) was infused intravenously over 2 min. The adductor pollicis twitch tension initially increased to a maximum and then, over time, gradually decreased. When the T1 response, after peaking, had again decreased to between 20% and 30% of control, the vecuronium infusion was terminated (fig. 1).

Blood samples for vecuronium concentration were collected before the first injection of vecuronium, immediately before neostigmine administration, and every 30 min for 2 h thereafter. Blood samples for neostigmine concentrations were collected before neostigmine was administered, and at 1, 2, 4, 8, 16, 30, 60, 90, 120 and 240 min thereafter. The volunteers remained anesthetized, and the target central body temperature was maintained until the final blood sample was drawn. Anesthesia was then discontinued, and the hypothermic subjects were actively rewarmed using a forced-air blanket (Bair Hugger).

Blood samples were heparinized, placed in ice, centrifuged and acidified within 1 h, and stored at −30°C. Plasma concentrations of vecuronium and its principal metabolite, 3-desacetylvecuronium, were determined using a capillary gas chromatographic assay.[6] This assay has a coefficient of variation of 4–15% and is linear over the concentration range 5–5,000 ng/ml. Because 3-desacetylvecuronium was likely to be present in significant amounts and has neuromuscular blocking potency (83% that of vecuronium), we needed to account for its effect during the steady state infusion.[7] Consequently, we defined a new variable, "effective vecuronium concentration," which is the sum of vecuronium and 3-desacetyulvecuronum (multiplied by 0.83) concentrations. Plasma concentrations of neostigmine were determined using high-performance liquid chromatography.[8] This assay separates the parent compound from metabolites, is sensitive to 10 ng/ml, is linear in the concentration range 10–1,000 ng/ml, and has a coefficient of variation of 5%.

The onset time was defined as the time from injection of neostigmine until maximum effect was achieved. The maximum effect of neostigmine-induced reversal was determined as the greatest observed increase in twitch tension as a proportion of the potential increase of T1 control given by the formula:

Maximum effect(%) =

$$100 \times (T1_{neo} - T1_{pre})/(100 - T1_{pre})$$

where $T1_{neo}$ is the twitch tension at peak reversal, and $T1_{pre}$ is the twitch tension at the time of neostigmine administration (fig. 1). Duration of action of neostigmine was defined as the time from injection of the drug until the twitch tension had decreased to 50% of maximum effect (fig. 1).

Values for onset, duration, and maximum effect of neostigmine, obtained during normothermia and mild hypothermia, were compared using Wilcoxon signed-rank test. "Effective vecuronium concentrations" for the 30-min intervals over the 2 h following administration of neostigmine were compared by repeated-measures analysis of variance (ANOVA). Averaged individual values for vecuronium concentration obtained during normothermia and mild hypothermia were compared using Wilcoxon signed-rank test. Values are expressed as median

Table 1. Neuromuscular Responses following Neostigmine during Normothermia (Norm) and Hypothermia (Hypo)

Subject	Temperature (°C)		Maximum T1 Effect (%)		Reversal Onset for T1 (min)*		Reversal Duration for T1 (min)		Maximum TOF Ratio		Reversal Onset for TOF (min)*		Reversal Duration for TOF (min)	
	Norm	Hypo	Norm	Hypo	Norm	Hypo	Norm	Hypo	Norm	Hypo	Norm	Hypo	Norm	Hypo
1	36.8	34.2	86	80	4.2	5.9	70	85	46	48	4.9	5.5	38	41
2	36.7	34.1	61	92	4.4	4.8	115	58	52	42	5.1	9.1	58	83
3	36.8	34.3	59	70	4.2	5.9	54	64	50	44	4.3	4.5	28	85
4	36.8	34.3	100	97	4.2	5.9	59	54	49	45	4.0	4.9	55	52
5	36.7	34.4	79	74	4.0	4.9	51	47	57	50	3.6	4.3	24	43
6	36.8	34.3	88	114	5.1	5.5	66	104	73	74	3.4	5.1	52	32
7	36.7	34.2	63	67	5.8	6.1	50	80	49	52	5.1	5.1	NA	NA
Median	36.8	34.3	77	85	4.6	5.6	66	70	54	51	4.3	5.5	43	56

T1 is the amplitude of the first response in a train-of-four (TOF) sequence and is expressed as a percentage of the control T1, which was obtained before administration of vecuronium.

* $P < 0.05$ normothermia *versus* hypothermia.

NA = study terminated before value determined.

(range) unless otherwise stated. Statistical significance was inferred at $P < 0.05$.

Pharmacokinetic Analysis

Mixed-effects population models (NONMEM) were fit to the neostigmine plasma concentration data using a model-building approach.[9,10] This approach defines a single basic model of typical values (population means) for the pharmacokinetic parameters. Initially, two- and three-compartment models were compared to determine which was the more appropriate. The parameters for structural models with two compartments were plasma clearance (Cl), distributional clearance (Q2), and volumes of the central (V1) and peripheral (V2) compartments. Structural models with three compartments had the additional parameters, slow distributional clearance (Q3) and volume of a deep peripheral compartment (V3). The volume of distribution at steady state (V$_{ss}$) was the sum of V1 and V2 for two-compartment models and the sum of V1, V2, and V3 for models with three compartments.

Variations in each individual from the basic model were defined by the use of a variable number of additional, user-defined, "interindividual variability parameters" (etas). Each η (η) defined a degree of variability in one or more of the basic parameters. For instance, clearance was modeled as:

$$Cl = Cl_{typical} \cdot \exp(\eta)$$

where Cl is the value for an individual, Cl$_{typical}$ is the typical value for the population, and η is a normally distributed random variable with mean zero. Both the basic model and the interindividual variability can also be wholly or partially modeled as functions of physiologic covariates, the aim being to reduce the residual degree of interindividual variability.

Improvements in three criteria were used to determine whether additional parameters should be incorporated into the model. These criteria were goodness of fit (-2 log likelihood), determinable precision for all parameters, and visual acceptability. We first compared models with two or three compartments and then tested models with two, three, or four η terms. Then we evaluated models with parameters that were weight normalized or not weight normalized. Finally, we tested four additional parameters, each permitting the following estimates to vary between the normothermic and hypothermic states: plasma clearance (Cl), intercompartmental clearance (Q2Q3), central volume of distribution (V1), and volume of distribution at steady state (V$_{ss}$).

Results

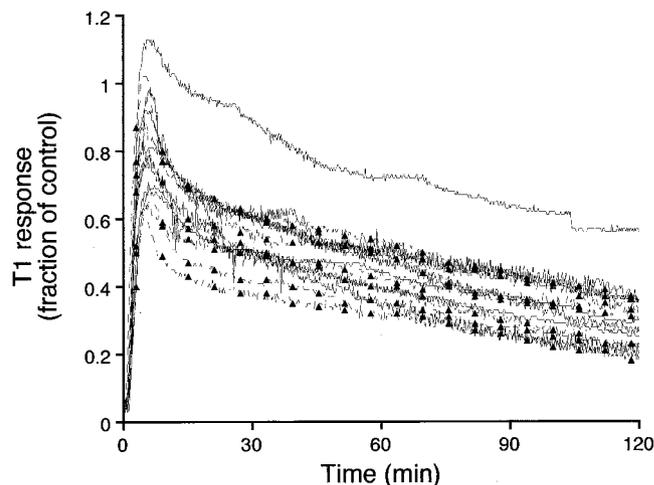The volunteers, 1 woman and 6 men, were aged 23–39 yr and weighed 58–79 kg. The time to stabilize body

Fig. 2. Neuromuscular response data following neostigmine administration for all subjects. Neostigmine, 70 μg/kg, was administered at time zero. T1 control was the amplitude of the fist response (T1) in the stable train-of-four sequence recorded just before the first administration of vecuronium. Data for normothermia are identified by the dashed lines and solid triangles.
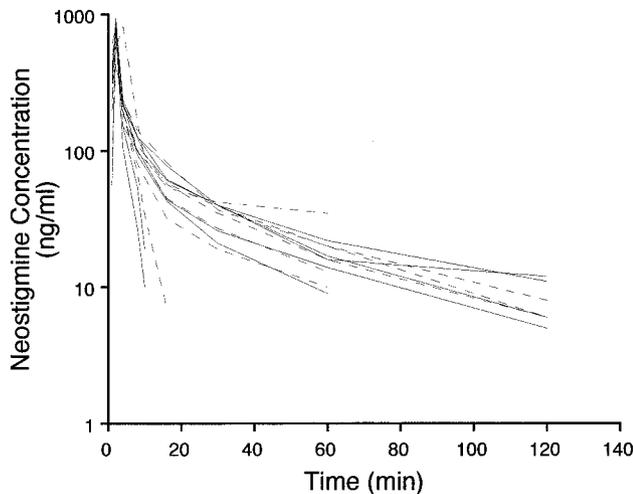
Fig. 3. Plasma concentration *versus* time data for neostigmine in normothermic (dashed lines) and hypothermic volunteers (solid lines).

temperature and adductor pollicis twitch tension, before administration of neostigmine, was 171 (120–250) min during normothermia and 173 (125–225) min during hypothermia.

The T1 response before neostigmine administration was 5% (4–6%) in both groups. The neuromuscular responses to neostigmine at normothermia and hypothermia are shown in table 1 and figure 2. The maximum effect of neostigmine on T1 and TOF responses at normothermia and hypothermia were similar. The time to maximum effect for both T1 and TOF was prolonged by hypothermia compared to normothermia ($P < 0.05$). The duration of action of neostigmine (for both T1 and TOF) was similar during normothermia and hypothermia.

Neostigmine concentrations over time are shown in figure 3. A model with three compartments was favored over one with two, and the pharmacokinetics were not weight related. The model building process is summarized in table 2. The parameters of the pharmacokinetic model are shown in table 3. As a demonstration of the fit of the model, the observed *versus* predicted neostigmine concentrations are shown in figure 4. The central volume of distribution (V1) decreased 38% with hypothermia compared to normothermia (table 3).

By repeated-measures ANOVA, the effective plasma concentrations of vecuronium did not change during the 2-h infusion period following administration of neostigmine (fig. 5). The effective plasma concentration of vecuronium producing 95% block during hypothermia, 236 ± 80 ng/ml, and normothermia, 272 ± 92 ng/ml, were not significantly different.

Discussion

In this study, we investigated the pharmacokinetics of neostigmine during mild hypothermia, and the ability of

Table 2. Building the Pharmacokinetic Model for Neostigmine

Elements Tested	Objective Function	Model No.
Compartments and weight relation		
Two compartments (Cl, Q2, V1, V2) not weight related	819.482	1
Two compartments (Cl, Q2, V1, V2) weight related	826.266	2
Three compartments not weight related*	777.81	3
Three compartments weight related	778.626	4
Model 3 is optimal at this stage.		
The eta model		
Model 3 + eta on Cl	777.811	5
Model 3 + eta on Q2 and Q3	779.012	6
Model 3 + eta on V1	779.016	7
Model 3 + eta on V2 and V3	779.009	8
Adding etas did not improve model fit.		
Effect of temperature		
Model 3 + temperature on Cl	776.920	9
Model 3 + temperature on Q2Q3	777.284	10
Model 3 + temperature on V1*	769.859	11
Model 3 + temperature on V2V3	773.960	12
Model 3 + temperature on V1 and V2V3	768.828	13
Model 3 + temperature on Cl and V1	769.859	14
Model 3 + temperature on Q2Q3 and V1	768.399	15
Model 11 is the final optimal model.*		

Cl is plasma clearance of neostigmine, Q2 is the distributional clearance, and Q3 is the slow distributional clearance. V1, V2, and V3 are the volumes of distribution for neostigmine in compartments 1, 2, and 3, respectively.

the drug to antagonize a steady state neuromuscular block produced by a constant-rate infusion of vecuronium in hypothermic and normothermic volunteers.

We did not find any change in maximum effect of neostigmine with hypothermia. This finding is supported by data obtained in cat studies, in which the neostigmine dose needed to achieve 50% reversal of steady state 90% block induced by pancuronium or d-tubocurarine remained constant over the temperature range 28–41°C.[11,12] However, any change with temperature in the dose needed to obtain the maximum effect of neostigmine may have been obscured by our study design. To study maximum effect and to increase the time of detection of neostigmine in plasma, a large dose of neostigmine (70 μg/kg) was administered. This dose was likely sufficient to produce the maximum possible reversal of the vecuronium-induced block in both normothermic and hypothermic volunteers.[13] The administration of a smaller dose might have revealed a temperature-related effect. In the cat, however, different doses

Table 3. Pharmacokinetic Parameters for Neostigmine (SE in Parentheses)

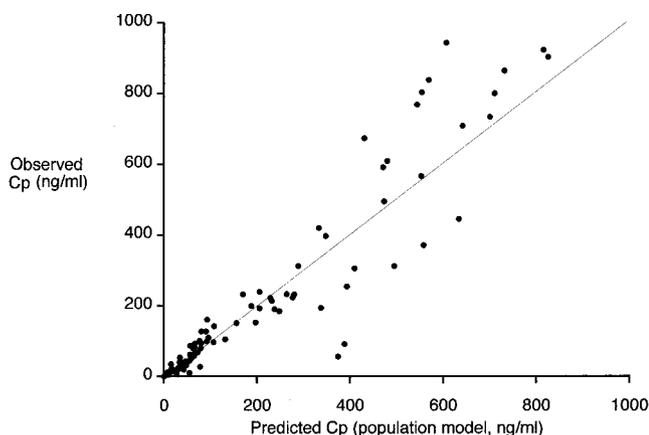| Variable	Normothermia	Effect of Hypothermia
Cl (ml/min)	696 (175)	None
Q2 (ml/min)	1,480 (175)	None
Q3 (ml/min)	556 (47)	None
V1 (ml)	5,590 (926)	Decrease by 38% (14)
V2 (ml)	15,000 (3,539)	None
V3 (ml)	110,000 (73,900)	None

Fig. 4. Observed *versus* predicted concentrations of neostigmine generated from optimal pharmacokinetic model (table 2). The solid line is the line of identity.

had similar maximum effect at different body temperatures.[11,12]

The duration of action of neostigmine as an antagonist of vecuronium-induced block is dependent on both the rate of decrease of the plasma concentration and the interaction with the enzyme acetylcholinestearase.[14-16] During the interaction of neostigmine with acetylcholinesterase, a relatively stable carbamylated complex is formed.[14,15] Decarbamylation of this complex is slow, and the process has a half-life of approximately 30 min. Termination of the effect of neostigmine may be rate limited by decarbamylation of the neostigmine/enzyme complex rather than simply the decrease in the plasma concentration of neostigmine.[14-16] Hypothermia may slow the decarbamylation process, but this has been studied only *in vitro* and at temperatures of 30°C and below, and these results cannot be related directly to our study.[17] The lack of effect of hypothermia on neostig-
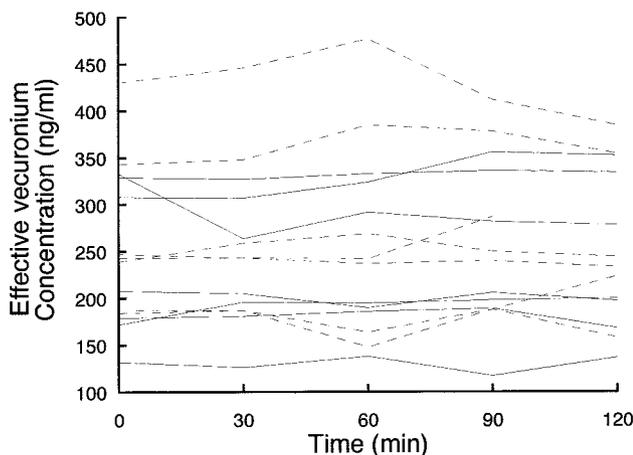

Fig. 5. Effective plasma concentrations of vecuronium *versus* time for the 2 h following neostigmine administration in normothermic (dashed lines) and hypothermic volunteers (solid lines). The effective concentration is the sum of vecuronium plus that of its active metabolite, 3-desacetylvecuronium. Concentrations of 3-desacetylvecuronium were multiplied by 0.83 to reflect its potency relative to vecuronium.[7]

mine Cl is consistent with its lack of effect on its duration of action.

In contrast to the lack of effect on duration of action and efficacy of neostigmine, hypothermia was associated with a delay in the onset of neostigmine. As determined by both T1 amplitude and TOF ratio, the onset of neostigmine was slower in the hypothermic subjects. The delayed onset with hypothermia was also observed in previous cat studies.[11,12] This observation may have been due to a hypothermia-related decrease in skeletal muscle blood flow.[18] However, the difference in onset time that we observed was less than 2 min and therefore was of only modest clinical significance.

We found a smaller V1 in hypothermia, and this might be expected to result in higher initial neostigmine concentrations and thus faster onset. However, other factors influence onset, including, as mentioned above, muscle blood flow. Our results suggest that other factors influencing drug effect at the neuromuscular junction predominate over any effect of the decreased V1 during hypothermia.

The Cl of neostigmine did not change with mild hypothermia. The value, which we obtained 696 ml/min, is similar to those reported previously, if an approximate adjustment for weight is made.[19-21] The only pharmacokinetic parameter that changed with hypothermia was V1, which decreased by 38%. It is difficult to interpret this finding. Because we did not obtain early arterial blood samples, we cannot have high confidence in our estimation of V1.[22,23] Consequently, we will not speculate further on this finding.

Some other aspects of our study design merit discussion. The duration of action of neostigmine was measured from injection of the drug until maximum effect had diminished by 50%, rather than until the twitch tension returned to its preneostigmine value. This method was used for two reasons. First, other investigators have used the technique.[5,24] Second, waiting until complete return to preneostigmine conditions would have required several additional hours of anesthesia, and we did not think this justifiable.

Our study design required administration of anesthesia for a total of approximately 15 h over the two study days. To minimize volunteer risk, we studied the minimum number of subjects we considered would give valid results. This raises the possibility that we failed to detect real hypothermia-related differences. A formal power calculation shows that for variables whose mean values were compared, the study had a greater than 90% power to detect a difference of 20% between means during normothermia and hypothermia at $P < 0.05$. For the variables' maximum effect (by T1 and TOF ratio) and T1 duration of action, the direction of change with hypothermia was three subjects in one direction and four in the other. For a statistically significant difference to have been detected, we would have had to study at least eight

more volunteers, and in all of them, the change of the variable with hypothermia would have had to be in the same direction, an unlikely possibility. In addition, the validity of our results is supported by their agreement with those from earlier animal experiments. Therefore, although the number of subjects was small, we consider our results and conclusions to be valid.

It is important to emphasize that this study was performed during a steady state neuromuscular block. We did not address the clinical situation in which the plasma concentration of neuromuscular blocking drug is decreasing when neostigmine is administered. In the clinical situation, reversal of neuromuscular block is dependent on two processes, the interaction of neostigmine with acetylcholinesterase and the inherent duration of action of the neuromuscular blocking drug.[3,16] Because hypothermia doubles the duration of action of vecuronium,[1] it is likely that the incidence of inadequate reversal will be increased.[2-4] Our results suggest that if hypothermia is associated with inadequate reversal of block, it is because of temperature-related effects on the pharmacology of the neuromuscular blocking drug, not the inherent efficacy of neostigmine.

In summary, we have found that mild hypothermia does not change the maximum effect or duration of action of neostigmine as an antagonist of steady state vecuronium-induced neuromuscular block.

References

1. Heier T, Caldwell JE, Sessler DI, Miller RD: Mild intraoperative hypothermia increases duration of action and spontaneous recovery of vecuronium blockade during nitrous oxide-isoflurane anesthesia in humans. ANESTHESIOLOGY 1991; 74: 815–9

2. Savarese JJ, Caldwell JE, Lien CA, Miller RD: Pharmacology of muscle relaxants and their antagonists, Anesthesia, 5th edition. Edited by Miller RD. New York, Churchill Livingstone, 2000, pp 412–90

3. Bevan DR, Donati F, Kopman AF: Reversal of neuromuscular blockade. ANESTHESIOLOGY 1992; 77:785–805

4. Bevan DR, Smith CE, Donati F: Postoperative neuromuscular blockade: A comparison between atracurium, vecuronium, and pancuronium. ANESTHESIOLOGY 1988; 69:272–6

5. Gencarelli PJ, Miller RD: Antagonism of org NC 45 (vecuronium) and

6. Furuta T, Canfell PC, Castagnoli KP, Sharma ML, Miller RD: Quantitation of pancuronium, 3-desacetylpancuronium, vecuronium, 3-desacetylvecuronium, pipecuronium and 3-desacetylpipecuronium in biological fluids by capillary gas chromatography using nitrogen-sensitive detection. J Chromatogr 1988; 427: 41–53

7. Caldwell JE, Szenohradszky J, Segredo V, Wright PMC, McLoughlin C, Sharma ML, Gruenke LD, Fisher DM, Miller RD: The pharmacodynamics and pharmacokinetics of the metabolite 3-desacetylvecuronium (ORG 7268) and its parent compound, vecuronium, in human volunteers. J Pharmacol Exp Ther 1994; 270:1216–22

8. De Ruyter MG, Cronnelly R: Reversed-phase, ion-pair liquid chromatography of quaternary ammonium compounds: Determination of pyridostigmine, neostigmine and edrophonium in biological fluids. J Chromatogr 1980; 183:193–201

9. Beal SL, Sheiner LB: NONMEM User's guide. San Francisco, University of California, 1979

10. Beal SL, Sheiner LB: Methodology of population pharmacokinetics, Drug Fate and Metabolism: Methods and Techniques. Edited by Garrett E, Hirsh J. New York, Marcel Dekker, 1985, pp 135–83

11. Miller RD, Roderick LL: Pancuronium-induced neuromuscular blockade, and its antagonism by neostigmine, at 29, 37, and 41°C. ANESTHESIOLOGY 1977; 46:333–5

12. Miller RD, Van Nyhis LS, Eger EI: The effect of temperature on a d-tubocurarine neuromuscular blockade and its antagonism by neostigmine. J Pharmacol Exp Ther 1975; 195:237–41

13. Magorian TT, Lynam DP, Caldwell J, Miller RD: Can early administration of neostigmine, in single or divided doses, alter the course of neuromuscular recovery from a vecuronium-induced neuromuscular blockade? ANESTHESIOLOGY 1990; 73:410–4

14. Barber HE, Calvey TN, Muir KT: The relationship between the pharmacokinetics, cholinesterase inhibition and facilitation of twitch tension of the quaternary ammonium anticholinesterase drugs, neostigmine, pyridostigmine, edrophonium and 3-hydroxyphenyltrimethylammonium. Br J Pharmacol 1979; 66: 525–30

15. Wilson IB, Harrison MA, Ginsberg S: Carbamyl derivatives of acetylcholinesterase. J Biol Chem 1961; 238:1498–500

16. Verotta D, Kitts J, Rodriguez R, Coldwell J, Miller RD, Sheiner LB: Reversal of neuromuscular blockade in humans by neostigmine and edrophonium: A mathematical model. J Pharmacokinet Biopharm 1991; 19:713–29

17. Roufogalis BD, Quist EE, Wickson VM: Effect of ligands on a discontinuous temperature dependence of the decarbamylation reaction of erythrocyte acetylcholinesterase. Biochim Biophys Acta 1973; 321:536–45

18. Dines KC, Mizisin AP, Jorge MC, Nunag KD, Kalichman MW: Effects of body and hindlimb temperature on laser Doppler blood flow and vascular conductance in rat sciatic nerve and skeletal muscle. J Neurol Sci 1997; 148:7–13

19. Cronnelly R, Stanski DR, Miller RD, Sheiner LB, Sohn YJ: Renal function and the pharmacokinetics of neostigmine in anesthetized man. ANESTHESIOLOGY 1979; 51:222–6

20. Fisher DM, Cronnelly R, Miller RD, Sharma M: The neuromuscular pharmacology of neostigmine in infants and children. ANESTHESIOLOGY 1983; 59:220–5

21. Morris RB, Cronnelly R, Miller RD, Stanski DR, Fahey MR: Pharmacokinetics of edrophonium and neostigmine when antagonizing d-tubocurarine neuromuscular blockade in man. ANESTHESIOLOGY 1981; 54:399–401

22. Viby-Mogensen J, Ostergaard D, Donati F, Fisher D, Hunter J, Kampmann JP, Kopman A, Proost JH, Rasmussen SN, Skovgaard LT, Varin F, Wright PM: Pharmacokinetic studies of neuromuscular blocking agents: Good clinical research practice (GCRP). Acta Anaesthesiol Scand 2000; 44:1169–90

23. Beaufort TM, Proost JH, Kuizenga K, Houwertjes MC, Kleef UW, Wierda JM: Do plasma concentrations obtained from early arterial blood sampling improve pharmacokinetic/pharmacodynamic modeling? J Pharmacokinet Biopharm 1999; 27:173–90

24. Miller RD, Van Nyhuis LS, Eger EId, Vitez TS, Way WL: Comparative times to peak effect and durations of action of neostigmine and pyridostigmine. ANESTHESIOLOGY 1974; 41:27–33