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The Effect of Neostigmine on Twitch Tension and Muscle Relaxant Concentration during Infusion of Mivacurium or Vecuronium

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Background: **An investigation suggested that neostigmine may not effectively antagonize mivacurium, presumably because neostigmine impairs mivacurium's metabolism. However, the effect of neostigmine on mivacurium's metabolism *in vivo* has not been reported. Therefore, the effect of neostigmine on neuromuscular function and plasma mivacurium concentrations during constant mivacurium infusion was determined.**

Methods: **Mivacurium was infused in five patients to maintain 90% depression of adductor pollicis twitch tension, then 50 μg/kg intravenous neostigmine was administered without altering the mivacurium infusion. Peak twitch tension after neostigmine, plasma cholinesterase activity, and mivacurium concentrations before and after neostigmine were measured. Five additional patients were given 50 μg/kg neostigmine to antagonize block due to continuous infusions of vecuronium.**

Results: **Neostigmine produced less antagonism of mivacurium (39 ± 11%) than of vecuronium (54 ± 9%, $P < 0.05$). Neostigmine decreased plasma cholinesterase activity and increased plasma concentrations of the *trans-trans* and *cis-trans* stereoisomers of mivacurium ($P < 0.05$).**

Conclusions: **Neostigmine is less effective at antagonizing the neuromuscular effect of mivacurium than that of vecuronium during constant infusion. Neostigmine increases plasma mivacurium concentrations, likely explaining its limited efficacy. Our results confirm that neostigmine impairs the metabolism of mivacurium *in vivo* and may explain the observation that neostigmine may not effectively antagonize mivacurium-induced block. (Key words: Antagonists, neuromuscular: neostigmine. Enzymes: cholinesterase; plasma. Neuromuscular relaxants, nondepolarizing: mivacurium.)**

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KAO *et al.* reported that, when 70 μg/kg neostigmine is given to antagonize profound mivacurium-induced neuromuscular block, twitch tension recovers slower than if no antagonist is administered.[1] They speculated that the prolonged antagonism after neostigmine resulted from neostigmine inhibiting plasma cholinesterase (the enzyme believed to be responsible for the metabolism of mivacurium[2]), thereby decreasing mivacurium's metabolism; however, plasma mivacurium concentrations were not measured. We[3] reported that administration of edrophonium during constant infusion of mivacurium increases concentrations of mivacurium's two potent stereoisomers, *cis-trans* and *trans-trans;* however, we did not determine whether a similar phenomenon occurs with neostigmine. To further understand the relationship between neuromuscular antagonists and mivacurium, we administered mivacurium by continuous infusion to determine the effect of neostigmine on mivacurium concentrations and twitch tension.

Methods

With approval from our local Institutional Review Board and after obtaining informed consent, we studied ten patients, ASA physical status 1 and 2, aged 18–46 yr, scheduled for elective surgery. Patients exceeding 130% of ideal body weight; those with renal, hepatic, neuromuscular, and/or electrolyte disorders; and those taking medication known to interfere with neuromuscular function were excluded.

After intravenous administration of 1–2 mg midazolam, anesthesia was induced with 3–4 μg/kg fentanyl and 2–3 mg/kg propofol. Tracheal intubation was performed without paralysis,[4] and ventilation was controlled to maintain normocapnia (end-tidal P_{CO_2} of 30–35 mmHg; Datex Ultima, Helsinki). Anesthesia was

maintained with 70% N_2O and 1% end-tidal isoflurane. Electrocardiogram, Sp_{O_2}, noninvasive blood pressure, and esophageal temperature were monitored continuously. Esophageal temperature was maintained between 35.5°C and 37.0°C.

After induction of anesthesia, the ulnar nerve was stimulated *via* subcutaneous needle electrodes at the wrist. Supramaximal stimuli of 0.2 ms duration were delivered in a train-of-four at 2 Hz every 12 s (Digistim II, Neuro Technology, Houston, TX). Preload was maintained at 200–400 g. The evoked twitch tension of the adductor pollicis muscle was measured using a calibrated force transducer (Myotrace, Houston, TX) and amplified (DC Bridge Signal Conditioner, Gould Electronics, Valley View, OH). Twitch tension was digitized (NB-M10-16, National Instruments, Austin, TX), displayed (Lab View, National Instruments), and recorded on-line (Centris 650, Apple Computer, Hayward, CA). In addition, a strip chart recorded the evoked twitch tension (TA240, Gould Electronics). End-tidal isoflurane concentration was stable for >20 min, and the first twitch response of each train (T1) was stable for >10 min (the control twitch tension) before the muscle relaxant was administered.

For five subjects, mivacurium was infused at 1–3 $\mu g \cdot kg^{-1} \cdot min^{-1}$. When twitch tension stabilized, the mivacurium infusion rate was adjusted, based on the Hill equation, targeting 90% twitch depression.[5] When twitch tension was stable at approximately 10% of the control value for >10 min and the mivacurium infusion rate was unchanged for >15 min, patients received atropine (25 μg/kg, not to exceed 1 mg) and 50 μg/kg neostigmine. The mivacurium infusion was continued unchanged throughout the remainder of the study.

After induction of anesthesia, 5 ml of venous blood was obtained to document normal values for plasma cholinesterase activity and dibucaine inhibition (SmithKline Beecham Clinical Laboratories, Van Nuys, CA). Venous blood samples (two 5-ml aliquots) were obtained before neostigmine (two samples separated by 10 min) and at 2, 4, 8, and 16 min after neostigmine. One aliquot was used to determine plasma cholinesterase activity, the other to determine mivacurium concentrations. To prevent mivacurium from degrading *in vitro,* phospholine iodide (1.25 mg in 100 μl of water) was added to these samples immediately; samples were iced within 1 min, and the plasma phase was separated and frozen within 1 h. Plasma cholinesterase activity was determined photometrically using acetylthiocholine as a substrate. Mivacurium concentrations were determined by high-pressure liquid chromatography using a modification of the technique described by Brown *et al.*[6] and a spectrofluorometric detector (RF-511PC, Shimadzu, Tokyo). The assay is sensitive to 5 ng/ml for each of the three stereoisomers and has a coefficient of variation ≤16% at that concentration; the assay is not affected by the presence of neostigmine.

The remaining five subjects underwent the same protocol except that vecuronium was the muscle relaxant and plasma cholinesterase activity and dibucaine inhibition were not determined. Vecuronium concentrations were determined by gas-liquid chromatography,[7] sensitive to 10 ng/ml with a coefficient of variation <15% at that concentration; the assay is not affected by the presence of neostigmine.

Peak twitch tension after antagonism and time to peak antagonism were determined. Antagonism was calculated as:

$$\text{Antagonism} = \frac{(\text{peak twitch tension after antagonism} - \text{baseline})}{(100\% - \text{baseline})},$$

where baseline is the twitch tension immediately before neostigmine administration. For example, if twitch tension recovered from 11% of control to 53% of control, antagonism was (53% − 11%)/(100% − 11%) = 47%. Antagonism of mivacurium and vecuronium and time to peak effect were compared using Student's *t* test for unpaired data.

To document that concentrations of each of the mivacurium stereoisomers were at steady-state before neostigmine administration, the two plasma concentrations obtained before neostigmine were compared using Student's *t* test for paired data. Mivacurium concentrations and plasma cholinesterase activity values after neostigmine were compared to the control values using repeated-measures analysis of variance. Plasma vecuronium concentrations were analyzed in a manner similar to that for mivacurium. Values are reported as mean ± SD. Statistical significance was accepted when $P < 0.05$.

Results

With mivacurium, the infusion rate to maintain 90% twitch depression was 2.2 ± 0.9 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (range 1.1–3.4 $\mu g \cdot kg^{-1} min^{-1}$). Twitch tension immediately before neostigmine administration was 10.2 ± 1.5%. Baseline values for plasma cholinesterase activity and dibucaine inhibition were normal for all subjects. After

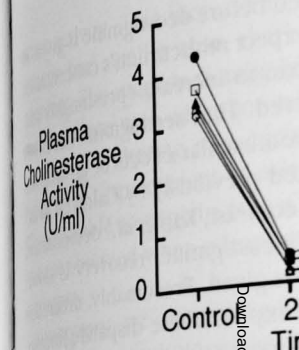

Fig. 1. Values for plasma cholinester
after antagonism of mivacurium with

neostigmine, plasma cholinestera
markedly ($P < 0.05$; fig. 1
With vecuronium, the infusion
twitch depression was 0.6 ±
(range 0.53–0.94 μg·kg⁻¹·min
immediately before neostigmine
10.2 ± 0.6%.
Antagonism of mivacurium (39
that of vecuronium (54 ± 9%, P
to peak antagonism was more ra
(4.2 ± 0.9 min; range 2.8–4.8 m
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0.05).
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Concentrations of each of those st
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determined in only four patients.
vary before or after neostigmine a
17 ng/ml before neostigmine, 1

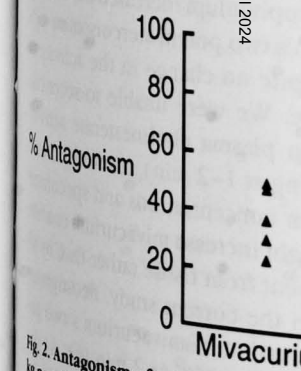


Fig. 2. Antagonism of mivacurium and
kg neostigmine.

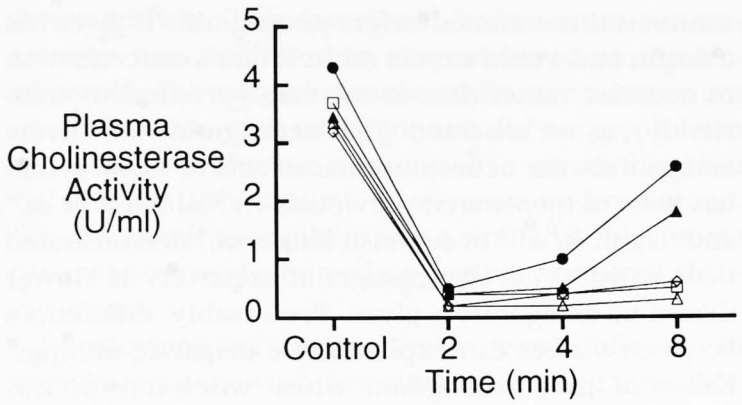


Fig. 1. Values for plasma cholinesterase activity before and after antagonism of mivacurium with 50 μg/kg neostigmine.

neostigmine, plasma cholinesterase activity decreased markedly ($P < 0.05$; fig. 1).

With vecuronium, the infusion rate to maintain 90% twitch depression was 0.65 ± 0.17 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (range 0.53–0.94 $\mu g \cdot kg^{-1} \cdot min^{-1}$). Twitch tension immediately before neostigmine administration was 10.2 ± 0.6%.

Antagonism of mivacurium (39 ± 11%) was less than that of vecuronium (54 ± 9%, $P < 0.05$; fig. 2). Time to peak antagonism was more rapid with mivacurium (4.2 ± 0.9 min; range 2.8–4.8 min) compared to vecuronium (7.0 ± 2.2 min; range 3.4–9.4 min, $P < 0.05$).

Concentrations of each of the mivacurium stereoisomers did not vary before neostigmine administration. Concentrations of each of these stereoisomers increased after neostigmine ($P < 0.05$; figs. 3 and 4). Because of sampling difficulties, vecuronium concentrations were determined in only four patients. These values did not vary before or after neostigmine administration: 106 ± 17 ng/ml before neostigmine, 107 ± 18 ng/ml at 2 min, 102 ± 20 ng/ml at 4 min, 106 ± 17 ng/ml at 8 min, and 102 ± 11 ng/ml at 16 min.

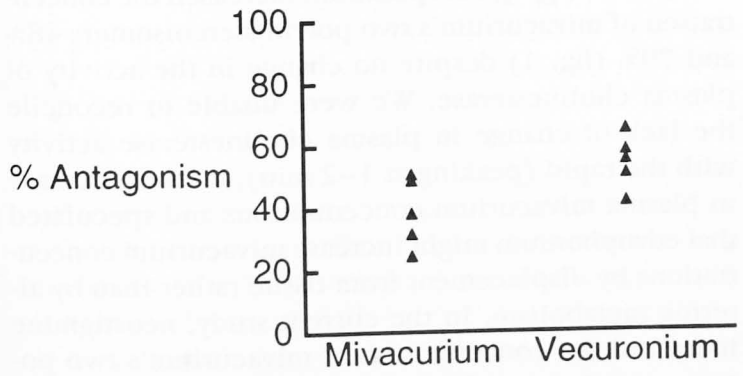
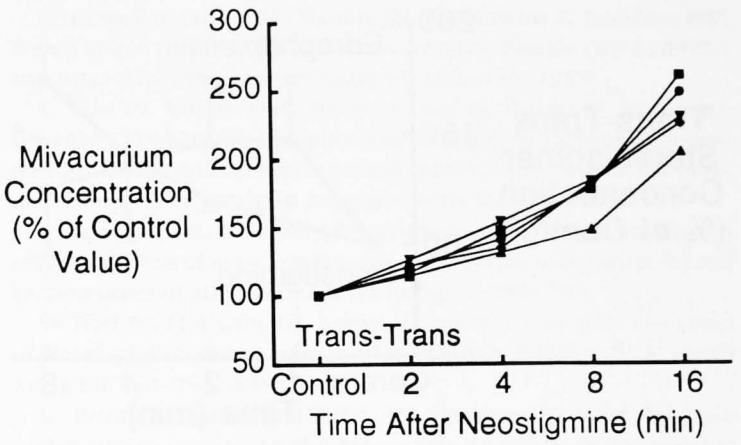
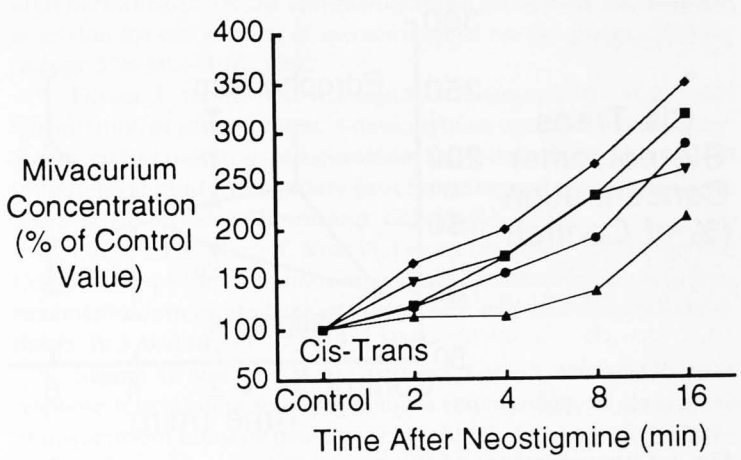


Fig. 2. Antagonism of mivacurium and vecuronium by 50 μg/kg neostigmine.





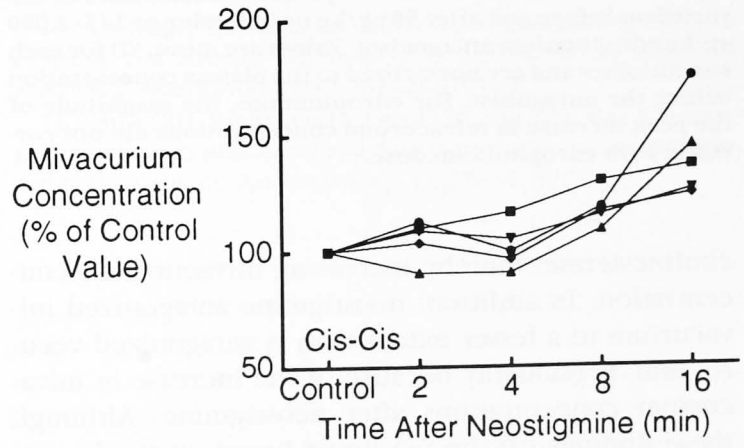


Fig. 3. Concentrations of the three stereoisomers of mivacurium for each individual patient before and after neostigmine antagonism. Values are normalized to the plasma concentration before neostigmine.

Discussion

Administration of neostigmine during constant infusion of mivacurium decreased the activity of plasma

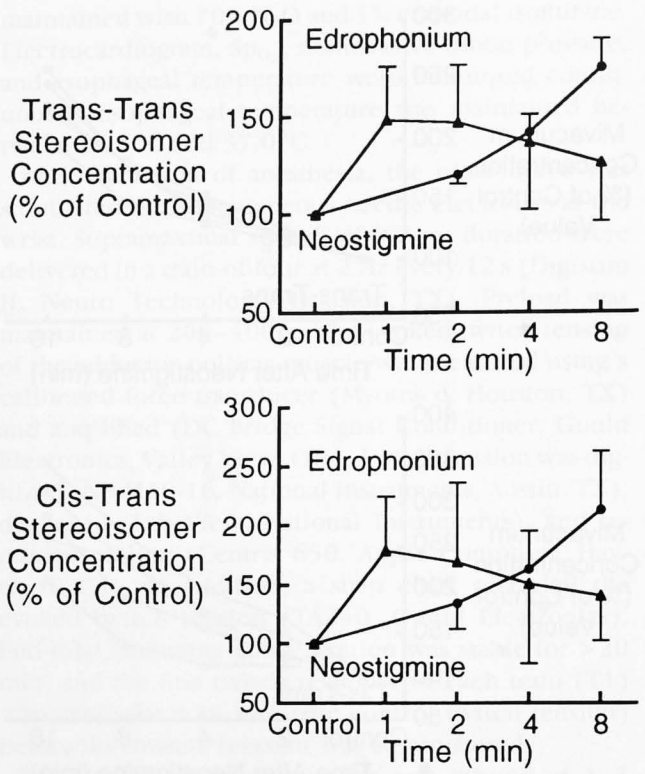


Fig. 4. Concentrations of the two potent stereoisomers of mivacurium before and after 50 μg/kg neostigmine or 125–2,000 μg/kg edrophonium antagonism. Values are mean, SD for each stereoisomer and are normalized to the plasma concentration before the antagonist. For edrophonium, the magnitude of the peak increase in mivacurium concentrations did not correlate with edrophonium dose.

cholinesterase, thereby increasing mivacurium's concentration. In addition, neostigmine antagonized mivacurium to a lesser extent than it antagonized vecuronium, presumably because of the increase in mivacurium concentrations after neostigmine. Although these findings can be predicted based on the known effects of neostigmine on plasma cholinesterase, they have not been demonstrated previously *in vivo*. In addition, our findings are consistent with Cook *et al.*'s observation that neostigmine decreased mivacurium's elimination *in vitro*.‖ Our results regarding antagonism of mivacurium by neostigmine must be considered cautiously; in clinical practice, mivacurium administration is discontinued before neostigmine is given. As a result, one would expect mivacurium's concentration to decrease rather than to increase (presumably transiently), as we observed. That neostigmine effectively antagonizes the neuromuscular effects of mivacurium has been demonstrated previously by Caldwell *et al.*[8] and Naguib *et al.*[9] In contrast, Kao *et al.*[1] demonstrated that, with 70 μg/kg neostigmine, recovery is slower than if no antagonist is given. Presumably, differences between studies can explain these disparate findings: Kao *et al.* gave neostigmine when twitch tension was 1–2% of control, whereas Caldwell *et al.* and Naguib *et al.* gave neostigmine when twitch tension recovered to 10% of control.

Our protocol was similar to that used to examine the dose-response relationship for antagonism of *d*-tubocurarine by edrophonium,[10] neostigmine,[11] and pyridostigmine.[11] In those studies, *d*-tubocurarine was infused to 90% steady-state block, after which single bolus doses of the antagonist were administered, and the infusion was continued unchanged. Under these experimental conditions, the neuromuscular effect of the antagonist was evaluated in the presence of a presumed constant plasma concentration of the muscle relaxant and unchanging anesthetic potentiation and P_{CO_2}. Although *d*-tubocurarine concentrations were not measured in those experiments, its pharmacokinetic characteristics and metabolic pathways suggest that its concentrations remained constant from antagonist administration to peak effect. In contrast, neostigmine's effect on the activity of plasma cholinesterase suggested (as demonstrated in the current study) that neostigmine administration would increase plasma mivacurium concentrations.

The results of the current study provide insight into an issue that we raised in a similar study of mivacurium's antagonism by edrophonium.[3] In that study, 125–2,000 μg/kg edrophonium increased the concentration of mivacurium's two potent stereoisomers 48% and 79% (fig. 4) despite no change in the activity of plasma cholinesterase. We were unable to reconcile the lack of change in plasma cholinesterase activity with the rapid (peaking at 1–2 min), marked increase in plasma mivacurium concentrations and speculated that edrophonium might increase mivacurium concentrations by displacement from tissue rather than by altering metabolism. In the current study, neostigmine increased the concentration of mivacurium's two potent stereoisomers 20% and 35% at 2 min (fig. 4) despite nearly complete inhibition of plasma cholines-

‖ Cook DR, Chakravorti S, Brandom BW, Stiller RL: Effects of neostigmine, edrophonium and succinylcholine on the in vitro metabolism of mivacurium: Clinical correlates (abstract). ANESTHESIOLOGY 77:A948, 1992.

terase activity. This increase in mivacurium concentrations was smaller than that observed after edrophonium. This suggests that the increase in mivacurium concentrations with edrophonium did not result from edrophonium altering mivacurium's metabolism but rather from displacement of mivacurium from tissue. We are unable to explain why a similar displacement was not observed when edrophonium antagonized vecuronium.

In summary, we antagonized constant infusions of muscle relaxants with neostigmine, finding less effective antagonism of mivacurium compared to that of vecuronium. After neostigmine, plasma mivacurium (but not vecuronium) concentrations increased, at least partially explaining this difference. Our results may explain Kao *et al.*'s observation that neostigmine delayed recovery from profound mivacurium-induced neuromuscular blockade. In addition, our results suggest that the role of neostigmine in the antagonism of mivacurium may differ from its role in the antagonism of other nondepolarizing muscle relaxants and deserves further study.

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