Pseudocholinesterase-mediated Hydrolysis Is Superior to Neostigmine for Reversal of Mivacurium-induced Paralysis In Vitro

H. S. Yang, M.D., Ph.D.,* N. Goudsouzian, M.D.† J. A. J. Martyn, M.D.‡

Background: The metabolic hydrolysis of mivacurium (and succinylcholine) is markedly impaired in the presence of hereditary or acquired defects of pseudocholinesterase. Clinical reports are conflicting as to the utility of anticholinesterases, in the reversal of mivacurium paralysis. In the current study, the role of exogenous cholinesterases and/or of anticholinesterase, neostigmine, in the reversal of deep mivacurium-induced paralysis, was studied. The rat phrenic-diaphragm preparation, in a fixed volume of Krebs solution, was chosen to eliminate the confounding effects on the dissipation of neuromuscular effects caused by hydrolysis, elimination, and redistribution of the drug.

Methods: In the phrenic-diaphragm preparation, mivacurium was administered to obtain >90% single twitch inhibition. Single twitch responses (0.1 Hz) were monitored for 60 min, after which the response to train-of-four stimulation was tested. The reversal of mivacurium by 0.5, 1.0, or 2.0 units/ml of (true) acetylcholinesterase, bovine pseudocholinesterase, or human plasma cholinesterase and by neostigmine, 0.1, 1.0, or 10.0 μg/ml was tested. The efficacy of human plasma cholinesterase, 1 unit/ml in combination with each of the above neostigmine concentrations, also was examined. The reversal of succinylcholine-induced paralysis by the acetylcholinesterase, bovine pseudocholinesterase, or human plasma cholinesterase (1 unit/ml) alone and in the presence of neostigmine (10.0 μg/ml) was additionally tested as a positive control. A train-of-four ratio > 0.75 was considered adequate reversal.

Results: Acetylcholinesterase was a poor hydrolyzer of mivacurium, as bioassayed by reversal of paralysis. Bovine pseudocholinesterase in concentrations of 0.5 and 1.0 units/ml did not effectively reverse single twitch and train-of-four responses by 60 min, but bovine pseudocholinesterase (2 units/ml) and all concentrations of human plasma cholinesterase did. Neostigmine alone, tested at all concentrations, was an incomplete reversal drug. Clinical or therapeutic concentrations (0.1 and 1.0 μg/ml) of neostigmine did not, but pharmacologic concentrations (10.0 μg/ml) did interfere with the efficacy of human plasma cholinesterase (1 unit/ml). Bovine pseudocholinesterase and human plasma cholinesterase equally reversed the effects of succinylcholine but acetylcholinesterase did not, whereas the addition of 10 μg/ml neostigmine to the enzymes inhibited the reversal of succinylcholine.

Conclusions: Human plasma cholinesterase will reverse mivacurium more effectively than bovine or human pseudocholinesterase, but both will effectively reverse succinylcholine. Acetylcholinesterase has no effects on either relaxant. The anticholinesterase neostigmine was an incomplete reversal drug. Pharmacologic concentrations of anticholinesterases do, while clinical or therapeutic concentrations do not, completely inhibit the metabolic activity of anticholinesterases. (Key words: Antagonists; anticholinesterase [neostigmine]; cholinesterases; pseudocholinesterases. Drug metabolism: mivacurium; succinylcholine. Interactions, drug; cholinesterases-mivacurium; cholinesterases-succinylcholine; mivacurium-neostigmine. Neuromuscular relaxants: mivacurium; succinylcholine.)

The neuromuscular effects of mivacurium and succinylcholine are terminated mostly by their hydrolysis of cholinesterases, particularly pseudocholinesterase. Thus, prolongation and/or potentiation of the neuromuscular effects of these drugs can occur when there is a genetic deficiency of the enzyme, or in the presence of drugs that inhibit the efficiency of the enzyme. The most commonly used classes of drugs that inhibit the enzymatic efficiency of the cholinesterases are the

* Postdoctoral Research Fellow in Anaesthesia.
† Associate Professor of Anaesthesia.
‡ Professor of Anaesthesia, Harvard Medical School, Massachusetts General Hospital; Associate Director of Anaesthesia, Shriners Burns Institute.

Address reprint requests to Dr. Martyn: Director, Clinical and Biochemical Pharmacology Laboratory, Department of Anaesthesia, Massachusetts General Hospital, 52 Fruit Street, Boston, Massachusetts 02114.

Address electronic mail to: martyn@anes.mgh.harvard.edu.


Anesthesiology, Vol 84, No 4, Apr 1996
REVERSAL OF CHOLINESTERASE-HYDROLYZABLE RELAXANTS

The purpose of this study was to determine the interaction of cholinesterases and/or anticholinesterase, neostigmine on mivacurium-induced profound (>90%) neuromuscular paralysis. The use of *in vitro* phrenic nerve-diaphragm preparation of a rat, in a fixed bath volume of Krebs solution, eliminated the endogenous metabolic clearance, distribution, and organ-based elimination components of dissipation of neuromuscular effect of the drugs occurring *in vivo*; the role of cholinesterases, anticholinesterases, and their interaction in the reversal of deep mivacurium-induced paralysis was thus more clearly defined.

Methods

**Animals, Preparation, and Drugs**

The protocol was approved by the Institutional Animal Care Committee at the Massachusetts General Hospital. Male Sprague-Dawley rats (*n* = 100; Charles River Breeding Laboratory, Wilmington, MA) weighing 100 g were studied. Each subgroup consisted of five animals. The animals were anesthetized with 40 mg/kg intraperitoneal pentobarbital sodium (Abbott Laboratories, Chicago, IL). The left hemidiaphragm, with the phrenic nerve attached, was extracted within 5 min. After adequate trimming, the neuromuscular preparation was attached to a glass chamber containing 10 ml Krebs solution at room temperature with 5% CO₂ and 95% O₂ continuously bubbled through the solution. The Krebs solution consisted of: NaCl 118; KCl 5; CaCl₂ 2.5; NaHCO₃ 30; KH₂PO₄ 1; MgSO₄ 1; and glucose; 11 mm. pH was maintained at 7.4 when aerated with a mixture of 95% O₂ and 5% CO₂ (vol/vol%).

Neostigmine was used as the prototypical anticholinesterase drug. The cholinesterase enzyme used were (true) acetylcholinesterase, bovine pseudocholinesterase, and human plasma cholinesterase. Acetylcholinesterase, bovine pseudocholinesterase, and neostigmine bromide were purchased from Sigma Chemical (St. Louis, MO). Human plasma cholinesterase was obtained from Pharmavene (Gaithersburg, MD).

**Drug Administration and Evoked Neuromuscular Responses**

Square wave pulses were delivered to the phrenic nerve, at supramaximal voltage by a Grass 88 Stimulator with an SIU 5 isolation unit (Grass Instruments, Quincy, MA). Evoked twitch height was measured *via* a pre-...
calibrated Grass FTO3 force-displacement transducer and recorded on a Western Graphp tech polygraph 4700 (Irvine, CA). Each preparation was allowed to equilibrate for at least 20 min until a stable, single twitch and train-of-four (TOF; 2 Hz/2 s) response was established. After stabilization of twitch tension, an approximate 90–95% inhibition of twitch tension (5–10% of baseline twitch height) by mivacurium or succinylcholine was achieved by incremental dose technique. The response to each dose was considered stable when four or five evoked twitch responses of the same height were obtained. Maximal twitch inhibition was attained in approximately 30 min. After maximal twitch suppression, single twitch stimuli were used for 60 min, after which the TOF ratio was tested. The groups, and protocol for drug administration, including concentrations, are summarized in Table 1. In all experiments, the saline, acetylcholinesterase, bovine pseudocholinesterase, or human plasma cholinesterase and/or neostigmine were added to the bath after maximal twitch inhibition had occurred, after the total dose of neuromuscular relaxant (vide infra). After the end of the experiment, the drugs were washed multiple times with Krebs solution, and the TOF ratio was retested.

**Evaluation of Stability and Dilutional Effects (Group 1)**

After a stable baseline single twitch tension was achieved, the TOF data were assessed. Single twitch stimuli were then continued for 90 min, after which TOF ratios were again determined. These tests confirmed the viability and stability of the preparation for this duration. In a separate set of neuromuscular preparations, after achieving more than 90% inhibition of the twitch with mivacurium (in approximately 30 min), 1 ml saline was added to the bath (time 0) and allowed for 60 min. Because the maximal volume of cholinesterase or neostigmine added to the bath was less than 1 ml, these control experiments tested the role of dilution on the reversal of mivacurium-induced paralysis.

**Interaction of Mivacurium with Cholinesterases (Groups 2–4)**

After mivacurium induced greater than 90% paralysis, acetylcholinesterase, bovine pseudocholinesterase, or human plasma cholinesterase, at concentrations of 0.5, 1.0, or 10.0 units/ml, were added to the bath (table 1, groups 2–4). The enzymatic efficiency of the cholinesterases, measured as units per milliliter, is standardized by their ability to metabolize acetyl or butyryl thiocholine. A standardized unit of each of the esterase enzyme hydrolyzes 1.0 μm butyryl thiocholine per minute at pH 7.4 and 20°C. After the addition of the enzyme, single twitch responses were monitored for 60 min followed by TOF stimulation.

**Interaction of Neostigmine with Human Plasma Cholinesterase (Groups 5–6)**

The utility of neostigmine alone (0.1, 1.0, or 10.0 μg/ml = 0.3, 3, and 30 nm respectively) as a reversal

*Mivacurium or ScH administered wherever indicated to produce > 90% twitch suppression (N = 5 for each subgroup). The same preparations were used for groups 7 and 8.*

<table>
<thead>
<tr>
<th>Group</th>
<th>Protocol bluronly</th>
<th>TOF at 0 and 90 min</th>
<th>(a) Mivacurium</th>
<th>(b) Mivacurium</th>
<th>(c) Mivacurium</th>
<th>(d) Mivacurium</th>
<th>(a) Mivacurium</th>
<th>(b) Mivacurium</th>
<th>(c) Mivacurium</th>
<th>(d) Mivacurium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Krebs’ solution only</td>
<td>Saline (1 ml, dilution effect)</td>
<td>ACHE 0.5 U/ml</td>
<td>ACHE 1.0 U/ml</td>
<td>ACHE 2.0 U/ml</td>
<td>ACHE 3.0 U/ml</td>
<td>ACHE 4.0 U/ml</td>
<td>ACHE 5.0 U/ml</td>
<td>ACHE 6.0 U/ml</td>
<td>ACHE 7.0 U/ml</td>
</tr>
<tr>
<td>Group 2</td>
<td>(a) Mivacurium</td>
<td>(b) Mivacurium</td>
<td>(c) Mivacurium</td>
<td>(d) Mivacurium</td>
<td>(e) Mivacurium</td>
<td>(f) Mivacurium</td>
<td>(g) Mivacurium</td>
<td>(h) Mivacurium</td>
<td>(i) Mivacurium</td>
<td>(j) Mivacurium</td>
</tr>
<tr>
<td>Group 3</td>
<td>(a) Mivacurium</td>
<td>(b) Mivacurium</td>
<td>(c) Mivacurium</td>
<td>(d) Mivacurium</td>
<td>(e) Mivacurium</td>
<td>(f) Mivacurium</td>
<td>(g) Mivacurium</td>
<td>(h) Mivacurium</td>
<td>(i) Mivacurium</td>
<td>(j) Mivacurium</td>
</tr>
<tr>
<td>Group 4</td>
<td>(a) Mivacurium</td>
<td>(b) Mivacurium</td>
<td>(c) Mivacurium</td>
<td>(d) Mivacurium</td>
<td>(e) Mivacurium</td>
<td>(f) Mivacurium</td>
<td>(g) Mivacurium</td>
<td>(h) Mivacurium</td>
<td>(i) Mivacurium</td>
<td>(j) Mivacurium</td>
</tr>
<tr>
<td>Group 5</td>
<td>(a) Mivacurium</td>
<td>(b) Mivacurium</td>
<td>(c) Mivacurium</td>
<td>(d) Mivacurium</td>
<td>(e) Mivacurium</td>
<td>(f) Mivacurium</td>
<td>(g) Mivacurium</td>
<td>(h) Mivacurium</td>
<td>(i) Mivacurium</td>
<td>(j) Mivacurium</td>
</tr>
<tr>
<td>Group 6</td>
<td>(a) Mivacurium</td>
<td>(b) Mivacurium</td>
<td>(c) Mivacurium</td>
<td>(d) Mivacurium</td>
<td>(e) Mivacurium</td>
<td>(f) Mivacurium</td>
<td>(g) Mivacurium</td>
<td>(h) Mivacurium</td>
<td>(i) Mivacurium</td>
<td>(j) Mivacurium</td>
</tr>
<tr>
<td>Group 7</td>
<td>(a) Succinylcholine</td>
<td>(b) Succinylcholine</td>
<td>(c) Succinylcholine</td>
<td>(d) Succinylcholine</td>
<td>(e) Succinylcholine</td>
<td>(f) Succinylcholine</td>
<td>(g) Succinylcholine</td>
<td>(h) Succinylcholine</td>
<td>(i) Succinylcholine</td>
<td>(j) Succinylcholine</td>
</tr>
<tr>
<td>Group 8</td>
<td>(a) Succinylcholine</td>
<td>(b) Succinylcholine</td>
<td>(c) Succinylcholine</td>
<td>(d) Succinylcholine</td>
<td>(e) Succinylcholine</td>
<td>(f) Succinylcholine</td>
<td>(g) Succinylcholine</td>
<td>(h) Succinylcholine</td>
<td>(i) Succinylcholine</td>
<td>(j) Succinylcholine</td>
</tr>
</tbody>
</table>

Anesthesiology. V 84, No 4, Apr 1996
REVERSAL OF CHOLINESTERASE-HYDROLYZABLE RELAXANTS

drug of mivacurium paralysis was evaluated (table 1, group 5). In a different set of neuromuscular preparations, the enzymatic effect of human plasma cholinesterase (1 unit/ml) was inhibited by neostigmine (0.1, 1.0, or 10 µg/ml) and their effects on mivacurium paralysis were evaluated (table 1, group 6). Based on a previous report of steady-state distribution volume of neostigmine of 0.7 l/kg, a 0.07 mg/kg dose of the same would result in a steady-state plasma concentration of 0.1 µg/ml. Thus, 0.1 µg/ml concentration was considered a clinical concentration. A tenfold increase (1.0 µg/ml) in the clinical concentration was selected based on previous report of neostigmine EC₅₀ of 1.0 µg/ml for inhibition of pseudocholinesterase. This concentration was considered a therapeutic concentration and was, therefore, not expected to cause total inhibition of human plasma cholinesterase. Ten times the EC₅₀ concentration was considered the pharmacologic dose (10 µg/ml).

Interaction of Succinylcholine with Cholinesterases with/without Neostigmine (Groups 7–8)

These studies were performed as positive controls for metabolic efficacy of cholinesterases. After inhibition of twitch tension by succinylcholine to more than 90% of baseline tension, acetylcholinesterase, bovine pseudocholinesterase, or human plasma cholinesterase was added and the effects of each on reversal observed for 60 min (group 7). The medium dose (1 unit/ml) of the cholinesterases was used. After termination of the experiments in group 7, the preparation was washed with Krebs solution at least 5 times with a 3-min interval between each wash. The twitch tension recovered to control responses, and was stable for approximately 10 min. Therefore, succinylcholine was added to the bath to induce more than 90% twitch depression. The effect of neostigmine (10 µg/ml) together with acetylcholinesterase, bovine pseudocholinesterase, or human plasma cholinesterase (1 unit/ml) on recovery of twitch tension was then observed for 60 min. Thus, experiments in group 8 were performed in the same preparations as that used for group 7.

Statistical Analysis

Data are presented as mean ± SD. Train-of-four ratio greater than 0.75 was considered adequate reversal of neuromuscular transmission. A TOF of 0.75 was chosen, based on a previous report that a TOF of 0.70 in the adductor pollicis may not truly reflect complete recovery from paralysis in other muscles. The recovery pattern of a single twitch was also compared between groups. One-way analysis of variance for repeated measures and Ryan-Einot-Gabriel-Welch multiple range tests were used to compare differences between groups. A P < 0.05 value was considered significant.

Results

Stability of the Phrenic-diaaphragm Preparation
The twitch tension at the end of 90 min was the same as that at time 0. The TOF ratios at 0 and 90 min (no mivacurium group) were 0.97 ± 0.02 and 0.96 ± 0.01, respectively (table 2, group 1a). The mean concentration of mivacurium required for 90–95% twitch inhibition for all experimental groups (group 1b–group 6c) was approximately 3.7 ± 0.05 µg/ml (range 3.0–5.0 µg/ml). In group 1b, where 1 ml saline was added at time 0 when the mivacurium-induced twitch inhibition was maximal, the twitch height did not change with time at the end of 60 min.

Cholinesterases on Mivacurium-induced Paralysis

Acetylcholinesterase at all concentrations had no effect on mivacurium-induced neuromuscular paralysis (table 2, group 2). The bovine pseudocholinesterase at lower concentrations of 0.5 and 1.0 units/ml did not completely reverse mivacurium effects, assessed by single twitch which did not reach near baseline levels (table 2, group 3). Thus, TOF of responses were not tested. The larger bovine pseudocholinesterase concentration of 2.0 units/ml, however, reversed the twitch height to >90% of baseline (<10% inhibition) at the end of 60 min and the TOF ratio to 0.86. All concentrations of human plasma cholinesterase tested were more effective than bovine pseudocholinesterase or acetylcholinesterase and resulted in complete reversal of single twitch and TOF ratios (table 2, group 4). The efficacy of the reversal was not increased by increasing the concentration (0.5 vs. 1.0 vs. 2.0 units/ml) of human plasma cholinesterase.

Neostigmine Alone and with Human Plasma Cholinesterase on Mivacurium-induced Paralysis

Although the three concentrations (0.1, 1.0, and 10 µg/ml) of neostigmine reversed the single twitch re-

Anesthesiology. V 84, No 4. Apr 1996
Table 2. Mivacurium Reversal with Cholinesterases and/or Neostigmine: Single Twitch Height (% of Control) and Train-of-Four Ratio

<table>
<thead>
<tr>
<th>Group 1. Controls</th>
<th>0 min</th>
<th>10 min</th>
<th>30 min</th>
<th>60 min</th>
<th>TOF Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Krebs' solution only</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0.96 ± 0.01</td>
</tr>
<tr>
<td>(b) 1.0 ml saline only</td>
<td>7 ± 1</td>
<td>5.0 ± 4</td>
<td>5.0 ± 4</td>
<td>5.0 ± 4</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group 2. ACHE</th>
<th>0 min</th>
<th>10 min</th>
<th>30 min</th>
<th>60 min</th>
<th>TOF Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) 0.5 U/ml</td>
<td>8 ± 2</td>
<td>6 ± 1</td>
<td>4 ± 2</td>
<td>4 ± 3</td>
<td>0.96 ± 0.01</td>
</tr>
<tr>
<td>(b) 1.0 U/ml</td>
<td>4 ± 2</td>
<td>3 ± 3</td>
<td>3 ± 3</td>
<td>3 ± 3</td>
<td>0.96 ± 0.01</td>
</tr>
<tr>
<td>(c) 2.0 U/ml</td>
<td>6 ± 2</td>
<td>5 ± 4</td>
<td>5 ± 3</td>
<td>5 ± 4</td>
<td>0.96 ± 0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group 3. BPChE</th>
<th>0 min</th>
<th>10 min</th>
<th>30 min</th>
<th>60 min</th>
<th>TOF Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) 0.5 U/ml</td>
<td>8 ± 4</td>
<td>14 ± 7</td>
<td>34 ± 20</td>
<td>66 ± 18</td>
<td>0.94 ± 0.01</td>
</tr>
<tr>
<td>(b) 1.0 U/ml</td>
<td>5 ± 4</td>
<td>16 ± 13</td>
<td>41 ± 22</td>
<td>69 ± 16</td>
<td>0.94 ± 0.01</td>
</tr>
<tr>
<td>(c) 2.0 U/ml</td>
<td>5 ± 2</td>
<td>24 ± 7</td>
<td>74 ± 14</td>
<td>94 ± 7</td>
<td>0.94 ± 0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group 4. HPChE</th>
<th>0 min</th>
<th>10 min</th>
<th>30 min</th>
<th>60 min</th>
<th>TOF Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) 0.5 U/ml</td>
<td>9 ± 5</td>
<td>49 ± 16</td>
<td>98 ± 6</td>
<td>100 ± 1</td>
<td>0.71 ± 0.09</td>
</tr>
<tr>
<td>(b) 1.0 U/ml</td>
<td>6 ± 2</td>
<td>51 ± 21</td>
<td>99 ± 3</td>
<td>100 ± 1</td>
<td>0.71 ± 0.09</td>
</tr>
<tr>
<td>(c) 2.0 U/ml</td>
<td>4 ± 2</td>
<td>52 ± 15</td>
<td>94 ± 4</td>
<td>100 ± 4</td>
<td>0.71 ± 0.09</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group 5. Neostigmine</th>
<th>0 min</th>
<th>10 min</th>
<th>30 min</th>
<th>60 min</th>
<th>TOF Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) 0.1 μg/ml</td>
<td>6 ± 2</td>
<td>59 ± 27</td>
<td>83 ± 15</td>
<td>93 ± 5</td>
<td>0.68 ± 0.05</td>
</tr>
<tr>
<td>(b) 1.0 μg/ml</td>
<td>6 ± 2</td>
<td>80 ± 19</td>
<td>94 ± 7</td>
<td>96 ± 6</td>
<td>0.68 ± 0.05</td>
</tr>
<tr>
<td>(c) 10.0 μg/ml</td>
<td>10 ± 3</td>
<td>89 ± 4</td>
<td>91 ± 6</td>
<td>93 ± 7</td>
<td>0.68 ± 0.05</td>
</tr>
</tbody>
</table>

Groups 1b through 6c received mivacurium to produce > 90% twitch suppression. Each subgroup consists of n = 5. Data are mean ± SD.

*P < 0.05, HPChE (0.5 or 1.0 U/ml) versus BPChE (0.5 or 1.0 U/ml) at 30 and 60 min.
†P < 0.001, HPChE (1 μg/ml) versus neostigmine (0.1 or 1.0 μg/ml) versus neostigmine alone or HPChE with neostigmine (10.0 μg/ml).

*sponses almost to baseline levels, the TOF ratios in all instances did not exceed 0.75 (table 2, group 5). That is, neostigmine alone in the absence of cholinesterase was an incomplete reversal drug, despite the presence of a visible (5-10%) twitch height before neostigmine administration. Concentrations of 0.1 and 1.0 μg/ml neostigmine with human plasma cholinesterase (1 unit/ml) did not decrease (inhibit) the efficiency of the enzyme as demonstrated by the reversal of single twitch and TOF ratios at the end of 60 min (table 2, group 6). In other words, the reversal of mivacurium by human plasma cholinesterase (1 unit/ml) was similar, both in the absence and presence of neostigmine, in concentrations of 0.1 and 1.0 μg/ml. Neostigmine 10 μg/ml with human plasma cholinesterase (1 unit/ml), however, reversed the single twitch to control levels, but the TOF ratios did not exceed 0.75. The reversal pattern of human plasma cholinesterase with 10 μg/ml of neostigmine was thus similar to that seen with each concentration of neostigmine alone (group 5).

**Discussion**

Many clinical situations exist where there is a hereditary or acquired deficit in efficacy of pseudocholinesterases resulting from pseudocholinesterase deficiency. Pseudocholinesterases are characterized by deficiencies in the plasma of patients with pseudocholinesterase deficiency. Although pseudocholinesterase deficiency is uncommon, the potential for profound and prolonged muscle relaxation is of concern. In the presence of pseudocholinesterase deficiency, the use of drugs that require plasma pseudocholinesterase for their metabolism may require increased doses of the drug or alternative treatment options. The use of neostigmine or other cholinesterase inhibitors in patients with pseudocholinesterase deficiency may be contraindicated due to the risk of excessive muscle relaxation.

**Reversal of Succinylcholine by Cholinesterases with and without Neostigmine**

A dose of 20 μg (or 2 μg/ml concentration in the bath) of succinylcholine resulted in an approximate 90-95% twitch suppression in all groups with 15 min of administration. In the presence of acetylcholinesterase, reversal of succinylcholine-induced paralysis was almost nonexistent at the end of 60 min (table 3, group 7). In contrast, equally rapid recovery was observed with bovine pseudocholinesterase or human plasma cholinesterase (1.0 unit/ml) by 30 min; the single twitch and TOF were fully recovered by 60 min. The coadministration of neostigmine (10.0 μg/ml) negated any beneficial effects observed with the cholinesterases, evidenced as no change in twitch tension at the end of 60 min (table 3, group 8).

Anesthesiology, V 84, No 4, Apr 1996
terases resulting in prolonged recovery from mivacurium or succinylcholine.\textsuperscript{1-3,5,25,27} § A common cause of acquired cholinesterase dysfunction is due to chemical compounds. Exposure to irreversible anticholinesterases (nerve gas or organophosphates, \textit{e.g.}, sarin, diisopropyl fluorophosphate) occurs during war, and after accidental or suicidal ingestion/inhalation of organophosphate insecticides.\textsuperscript{5,6,27,28} Nerve gas also is increasingly used in terrorist attacks.\textsuperscript{29} In patients with myasthenia gravis, neostigmine and other anticholinesterases are administered for prolonged periods, which can result in toxicity and cholinergic crisis.\textsuperscript{10} High doses of reversible anticholinesterases occasionally are administered in wartime conditions as a prophylaxis against nerve gas exposure.\textsuperscript{30} In these conditions, there is a potential for these anticholinesterases to inhibit the metabolism of mivacurium or succinylcholine.

The \textit{in vitro} reports of the actions of anticholinesterases during mivacurium paralysis have been quite conflicting. Although edrophonium does not inhibit plasma cholinesterase, increased plasma mivacurium concentrations during its continuous infusion have been demonstrated.\textsuperscript{31} The authors speculated that the increase in mivacurium concentrations was probably related to displacement of the relaxant from tissue binding sites by edrophonium. A similar increase in mivacurium concentrations also was observed after a 0.05-mg/kg dose of neostigmine; inhibition of pseudocholinesterase was attributed as the cause for the increase in concentration.\textsuperscript{32} Abduratiff compared edrophonium and neostigmine-induced (0.07 mg/kg) recovery from intense neurovascular block, and found that the reversal time with neostigmine was prolonged in comparison to edrophonium. He concluded that this difference was attributable to inhibition of pseudocholinesterase by neostigmine.\textsuperscript{18} Based on its volume of distribution,\textsuperscript{52} the resultant plasma concentrations of neostigmine at steady state in the latter two studies would be \(0.1\ \mu g/ml\). Other \textit{in vitro} studies, however, document facilitated recovery of mivacurium by neostigmine.\textsuperscript{12-15} § In view of the disparate observations on the interaction of anticholinesterases with mivacurium, our studies have attempted to distinguish effects of clinical, therapeutic, and pharmacologic concentrations of neostigmine on the reversal of mivacurium paralysis and quantify the inhibition of the esterases at each of these doses. The different combinations and concentrations of cholinesterases and neostigmine used in the experiments allowed us to determine the inhibitory or facilitatory effects of these drugs on mivacurium reversal. The use of \textit{in vitro} preparation with a fixed volume of a Krebs solution precluded the confounding effects of drug redistribution within the body, hydrolysis in plasma and of organ-dependent elimination; all these processes can occur simultaneously \textit{in vitro} and cause dissipation of drug effects.

The capacity of acetylcholinesterase to hydrolyze mivacurium has previously been demonstrated, albeit its efficacy is 30-fold lower than human plasma cholinesterase.\textsuperscript{3} The practical significance of this metabolic capacity of acetylcholinesterase to hydrolyze

### Table 3. Sch Reversal with Cholinesterases with/without Neostigmine: Single Twitch Height (% of Control) and Train-of-Four Ratio

<table>
<thead>
<tr>
<th>Cholinesterase</th>
<th>TOF Ratio</th>
<th>0 min</th>
<th>10 min</th>
<th>30 min</th>
<th>60 min</th>
<th>TOF Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 7: Sch with</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) AChE, 1.0 U/ml</td>
<td>0.96 ± 0.01</td>
<td>6 ± 3</td>
<td>9.0 ± 7</td>
<td>10 ± 6</td>
<td>17 ± 10</td>
<td>0.96 ± 0.01</td>
</tr>
<tr>
<td>(b) BChE, 1.0 U/ml</td>
<td>0.94 ± 0.01</td>
<td>6 ± 3</td>
<td>17 ± 14</td>
<td>75 ± 27*</td>
<td>95 ± 6.3</td>
<td>0.93 ± 0.03</td>
</tr>
<tr>
<td>(c) HPChe, 1.0 U/ml</td>
<td>0.94 ± 0.01</td>
<td>5 ± 3</td>
<td>53 ± 33†</td>
<td>88 ± 20*</td>
<td>95 ± 6.3</td>
<td>0.94 ± 0.02</td>
</tr>
<tr>
<td>Group 8: Sch with</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) AChE, 1.0 U/ml + neostigmine</td>
<td>0.86 ± 0.07</td>
<td>8 ± 1</td>
<td>4 ± 1</td>
<td>3 ± 1</td>
<td>3 ± 0</td>
<td>0</td>
</tr>
<tr>
<td>(b) BChE, 1.0 U/ml + neostigmine</td>
<td>0.84 ± 0.01</td>
<td>8 ± 2</td>
<td>6 ± 2</td>
<td>4 ± 1</td>
<td>4 ± 1</td>
<td>0</td>
</tr>
<tr>
<td>(c) HPCHe, 1.0 U/ml + neostigmine</td>
<td>0.84 ± 0.01</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
<td>0</td>
</tr>
</tbody>
</table>

Sch was administered to all groups to produce >90% twitch inhibition. Each subgroup consists of \(n = 5\); preparations from group 7 were also tested in group 8. Data are mean ± SD.

\(* P < 0.001\) BChE or HPChe versus AChE by 30 and 60 min.

† \(P < 0.05\) HPChe versus AChE by 10 min.
mivacurium was tested in the current pharmacodynamic study; the minimal reversal of mivacurium by acetylcholinesterase was confirmed. Another aim of the study was to compare the efficacy of bovine *versus* human plasma cholinesterase. Bovine pseudocholinesterase, in concentrations of 0.5 and 1.0 units/ml caused some reversal of single twitch, but not of the TOF. Bovine pseudocholinesterase of 2.0 units/ml, however, reversed single twitch and TOF ratio. All concentrations of human plasma cholinesterase were equally efficient, with full recovery of TOF. These findings indicate that the human plasma cholinesterase compared to bovine pseudocholinesterase was more specific to mivacurium.

Although several *in vivo* and *in vitro* studies have confirmed the facility of reversing deep (>90%) neuromuscular paralysis of intermediate- and long-acting relaxants by neostigmine, the studies on short duration relaxant, mivacurium, have been equivocal. In our study of mivacurium, all three concentrations of neostigmine reversed the single twitch height to above 90% of control, but the TOF ratios did not exceed 0.75. This observation is, therefore, consistent with some of these *in vitro* studies, but contrasts with other studies where adequate reversal of >90% paralysis was obtained with anticholinesterases. The discrepant results may be related to the *in vivo* nature of these studies where concomitant redistribution within the body, organ-based elimination, and pseudocholinesterase-dependent metabolism all may have occurred during the action of the reversal drug. Thus, the poor response to neostigmine alone in the bath may be related to the continued occupation of the receptor by the unmetabolized mivacurium that could not be redistributed, metabolized, or eliminated (vide infra). The ceiling effect of neostigmine alone on mivacurium reversal was thus confirmed.

Neostigmine (in the absence of pseudocholinesterase), may have affected reversal of neuromuscular transmission by additional mechanisms. Neostigmine, even at low concentrations (0.1 μg/ml), inhibits acetylcholinesterase and increases junctional levels of acetylcholine, which cannot be hydrolyzed or redistributed in the bath. The prolonged exposure to increased concentrations of acetylcholine may have caused a desensitization block of the receptor. At higher concentrations, neostigmine and other anticholinesterases also can inhibit the acetylcholine receptor, due to its allosteric “channel” binding properties. Desensitization block or potentiation of paralysis by anticholinesterases has been observed in humans, animals, and isolated cells and may account for the incomplete reversal with neostigmine. Whether, in fact, neostigmine in the bath will adequately reverse less profound paralysis was not tested.

The administration of neostigmine in combination with human plasma cholinesterase had a variable response on mivacurium paralysis. Concentrations of 0.1 and 1.0 μg/ml neostigmine with human plasma cholinesterase (1 unit/ml) did not enhance or retard the reversal of mivacurium when compared to human plasma cholinesterase alone. Thus, these concentrations of neostigmine did not significantly impair the enzymatic efficiency of human plasma cholinesterase. Our study is therefore consistent with the study of Cook et al. that the concentrations of 0.1 and 1 μg/ml do not completely inhibit the metabolic capacity of the pseudocholinesterases; even at 50% inhibition, human plasma cholinesterase can affect efficient mivacurium hydrolysis. The reversal (single twitch and TOF) of mivacurium when treated with human plasma cholinesterase and 10 μg/ml neostigmine (group 6c), was similar to that of neostigmine alone (group 5a–5c). The similarity in the reversal pattern thus indicates that the concentration of 10 μg/ml neostigmine inhibited the enzyme function totally and thus the effects of neostigmine alone were manifest. This experiment therefore characterizes the importance of clearance of mivacurium from the receptor by enzyme hydrolysis versus competitive clearance by acetylcholine in its reversal.

Thus, our studies on mivacurium reversal indicate that human plasma cholinesterase is more efficient than bovine pseudocholinesterase. Higher concentrations of bovine pseudocholinesterase are, however, equally effective. Acetylcholinesterase is ineffective. Neostigmine alone is a poor reversal drug, even in the presence of a twitch, especially if metabolic hydrolysis redistribution and organ-based elimination of the drug are absent. Our study also confirms that the usual clinical (0.1 μg/ml) or even ten times this concentration does not completely inhibit pseudocholinesterase and confirms the *in vitro* observations of Cook et al. Anther inference from our observations is that the trans and the trans-cis isomers of mivacurium having potent neuromuscular effects are also metabolized by the pseudocholinesterases. The observation of a full recovery with human plasma cholinesterase indicates that the cis-cis component, which is not hydrolyzed by plasma cholinesterase, has no significant neuromuscular blocking effect in vivo, and confirms the observations made in vivo.
REVERSAL OF CHOLINESTERASE-HYDROLYZABLE RELAXANTS

The studies using succinylcholine served as positive controls. Our study confirms previous observations that succinylcholine (as with mivacurium) is a poor substrate of acetylcholinesterase.1-5 Both bovine pseudocholinesterase and human plasma cholinesterase, in concentration of 1 unit/ml, however, were equally effective in the reversal of succinylcholine paralysis. This contrasts, therefore, with the different efficiencies of bovine pseudocholinesterase and human plasma cholinesterase in the metabolism of mivacurium. Neostigmine (10.0 μg/ml), as observed in our study with mivacurium, completely inhibited the enzymatic effects of bovine pseudocholinesterase and human plasma cholinesterase, evidenced as no reversal of succinylcholine.

The contrasting efficiencies of the reversal patterns of mivacurium and succinylcholine by human plasma cholinesterase and bovine pseudocholinesterase emphasize the point that the hydrolytic ability of one enzyme (e.g., bovine pseudocholinesterase) to metabolize a given drug (e.g., mivacurium) cannot be extrapolated to another drug (e.g., succinylcholine), just because the unit strength, based on butryrylcholine hydrolysis, is the same. Consequently, when comparisons are made of the efficiencies of these different cholinesterase enzymes, the importance of concentration, species of origin, and the substrate (drug) should be taken into consideration.

The clinical implications of this study are as follows: Exogenous true cholinesterase is not useful in the reversal of neuromuscular paralysis after succinylcholine or mivacurium. When prolonged mivacurium- or succinylcholine-induced neuromuscular paralysis is due to hereditary or acquired deficiency of plasma cholinesterase, the administration of neostigmine even in the presence of a demonstrable twitch may not reverse the paralysis. Neostigmine may be particularly ineffective after prolonged administration of cholinesterase-hydrolyzable relaxants or in the presence of excretory organ dysfunction, when rapid redistribution and elimination of the relaxant drug, respectively, cannot occur. Exogenous plasma cholinesterases, in contrast, would be effective. Such therapy may be particularly advantageous in many conditions where rapid reversal of mivacurium or succinylcholine-induced paralysis is a requisite. These clinical states include the early differential diagnosis of muscle relaxant versus other causes of paralysis in a neurologically injured patient, or prolonged paralysis in an ambulatory care setting. In a patient with homozgyous atypical plasma cholinesterase, paralysis lasting up to 6–8 h can result from a single dose even in the presence of normal renal function.6 In view of the enormous cost of human plasma cholinesterase, an alternative may be the use of bovine pseudocholinesterase, which in higher concentrations is equally effective for mivacurium or succinylcholine. The cost of 50 mg (300 units) of bovine pseudocholinesterase is $30, compared with $300 for human plasma cholinesterase.7 Although drugs such as insulin, heparin, and protamine from bovine, equine, porcine, or piscine sources are being used in humans, the clinical utility of pseudocholinesterases from these nonhuman sources is unknown.

References


