Carotid Body Chemoreceptor Function Is Impaired by Vecuronium during Hypoxia

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Background: Neuromuscular blocking agents reduce the human ventilatory response to hypoxia at partial neuromuscular block. It was hypothesized that vecuronium impairs carotid body chemoreceptor function during hypoxia.

Method: The effect of systemic administration of vecuronium on single chemoreceptor activity during hypoxia, as recorded from a single nerve fiber preparation of the carotid sinus nerve, was studied in seven mechanically ventilated New Zealand White rabbits during continuous thiopental anesthesia. During normoventilation, the isocapnic hypoxic chemosensitivity of the single carotid body chemoreceptor was measured at four levels of oxygenation; these measurements were repeated at six separate occasions; control recording before injection, after intravenous administrations of 0.1 mg and 0.5 mg of vecuronium, and then at three occasions during a 90-min recovery period. Chemoreceptor chemosensitivity during isocapnic hypoxia was expressed as a hyperbolic function: Chemoreceptor output (Hz) = a + b × PaO₂<sup>−1</sup> (mmHg).

Results: Chemosensitivity was reduced after both 0.1 mg and 0.5 mg vecuronium intravenous administration compared with control measurements; the hypoxic response curve was significantly depressed after both doses (P < 0.05). Notably, there was variation in the effect of vecuronium; some chemoreceptor preparations showed only minimal impairment, whereas some showed an almost abolished response to hypoxia. The chemosensitivity remained significantly depressed at 30 and 60 min but had recovered spontaneously at 90 min after 0.5 mg vecuronium.

Discussion: It is concluded that vecuronium depresses carotid body chemoreceptor function to a varying extent during hypoxia and that the depression recovers spontaneously. (Key words: Carotid body; hypoxemia; neuromuscular blocking agent; respiration; vecuronium.)

It has recently been shown that commonly used neuromuscular blocking agents reduce the poikilocapnic<sup>1</sup> and isocapnic hypoxic ventilatory response in humans,<sup>2,3</sup> whereas the hypercapnic ventilatory response is maintained.<sup>1,3</sup> It is now hypothesized that the effect on hypoxic responses may be caused by an interaction with carotid body chemosensitivity.<sup>2,4</sup> In the rabbit, vecuronium administered locally close to the carotid body caused a reduction in the phrenic nerve activity during systemic hypoxic challenges.<sup>3</sup> Furthermore, in vitro preparations of the carotid body have shown that the neuronal activity of the sinus nerve is reduced in the presence of acetylcholine receptor blocking agents.<sup>5</sup> Nicotinic and muscarinic reactive sites are demonstrated on the glomus cell, and these may be blocked by a nondepolarizing neuromuscular blocking agent, such as d-tubocurarine.<sup>6</sup> We have therefore suggested that the depressed response to hypoxia found in humans during partial neuromuscular block<sup>1,4</sup> and in the rabbit<sup>3</sup> most likely result from a direct effect on the peripheral chemoreceptor function. Hence, the objective of the present study was to examine the effect of systemic administration of vecuronium on single fiber afferent chemoreceptor activity during hypoxia in anesthetized rabbits.

Method

Animals and Anesthesia

The study was approved by the Local Animal Care and Use Committee of the Karolinska Institute, Stockholm, Sweden. Seven adult New Zealand White rabbits weighing 3860 ± 270 g (mean ± SD) were included. After an initial dose of intravenous thiopental, 75-100 mg, given
via a marginal ear vein, a tracheostomy was performed after an anterior midline skin incision. Mechanical ventilation (ET\textsubscript{CO}_2, 4.0-5.0%) was initiated at a rate of 33 breaths/min using a mixture of oxygen and air (F\textsubscript{O}_2, 0.30), whereas the inspired tidal volume was adjusted to maintain isocapnia throughout the experiment. Anesthesia was maintained with a continuous thiopental infusion of 10.5 ± 0.9 mg·kg\textsuperscript{-1}·h\textsuperscript{-1} (range, 9.4-12.0) given via a right femoral vein catheter. The right femoral artery was cannulated for continuous arterial blood pressure monitoring and arterial blood gas analysis (ABL 300 Laboratory\textsuperscript{®}, Radiometer, Copenhagen, Denmark). On-line analysis of inspired and expired oxygen and carbon dioxide concentrations were performed using a Datex 254 gas analyzer (Datex\textsuperscript{®}, Helsinki, Finland) attached via a gas sampling line at the tracheostomy tube. Body temperature was monitored with a standard rectal probe, and a temperature of 37.7 ± 0.6°C (range, 37.0-39.0) was maintained by a servo-controlled heating pad positioned under the animal.

**Chemoreceptor Recording and Experimental Procedure**

The trachea and esophagus were divided and retracted cranially to expose the left carotid bifurcation and carotid sinus. Under the microscope, the sinus nerve was sectioned proximally at its junction with the glossopharyngeal nerve and meticulously dissected toward the carotid body. Exposed tissue was covered with 5-10 ml of liquid paraffin, which filled a bath covering the carotid body, sinus nerve, and surrounding structures. The sinus nerve was desheathed and positioned on a grounded stainless steel plate. Small bundles of nerve fibers were manually split into fine strands until a single chemoreceptor action potential was identified from one pole of a bipolar platinum electrode. A piece of glossopharyngeal nerve of similar thickness as the sinus nerve was suspended between the plate and the other pole of the electrode.

A Digitimer Neurolog system (Digitimer, Welwyn Garden City, UK) was used for processing neuronal activities. The single chemoreceptor action potential was amplified (NL 100 AK, NL 104), filtered (NL 125), and transferred to a storage oscilloscope (Tektronics, Wilsonville, OR). A window height discriminator (NL 200) was set to trigger a standard pulse, the frequency of which was calculated over a preset time interval of 1-5 s (NL 304) using a ratemeter. Invasive arterial blood pressure (mmHg), tracheal O\textsubscript{2} and CO\textsubscript{2} concentrations (%), and the chemoreceptor output (Hz) were also continuously displayed on a monitor and stored on a computer hard disk for later analysis using Axotape software (Axon\textsuperscript{®}, USA).

The criteria for using a chemoreceptor signal and for subsequent analysis and inclusion of the data were the following: (1) random chemoreceptor firing pattern free of baroreceptor interference, (2) a resting firing frequency of 2-10 Hz during normoxia (F\textsubscript{O}_2, 0.21-0.25), (3) increasing firing frequency to approximately 20-30 Hz during moderate hypoxia (F\textsubscript{O}_2, 0.10-0.15) and decreasing frequency during hyperoxia (F\textsubscript{O}_2 > 0.40), and (4) consistent configuration of the action potential throughout the experimental procedure.

The chemosensitivity was repeatedly measured by a series of step reductions in F\textsubscript{O}_2 from 0.25 to approximately 0.10; each series targeted within four different levels of oxygenation (P\textsubscript{a}O\textsubscript{2}, 40, 55, 80, and 110 mmHg) during isocapnia. After the step change in F\textsubscript{O}_2, a 3-4 min period passed until the end-tidal O\textsubscript{2} concentration and chemoreceptor output had reached a plateau.

Thereafter arterial blood samples were drawn at the end of a 60-s period of steady-state chemoreceptor activity for immediate blood gas analysis. Four or five blood gas measurements were made to verify the desired levels of oxygenation. The chemoreceptor activity was recorded simultaneously and calculated as the mean frequency during a 45-s period after blood sampling.

The isocapnic hypoxic chemosensitivity of the chemoreceptor was described as a hyperbolic function as previously described:

\[ \text{Chemoreceptor output (Hz)} = a + b \left( \frac{\text{P}_{a}O_2}{1}\right)(\text{mmHg}) \]

For each experimental state and for each animal, chemoreceptor output and corresponding P\textsubscript{a}O\textsubscript{2} values were fitted into the equation using least square regression analysis.

The measurements of hypoxic chemoreceptor responses were made at six different occasions; each occasion was separated by a 30-min interval. Control recordings started 3 min after a femoral vein injection of 3 ml normal saline. After a 30-min pause, 0.1 mg vecuronium, diluted in 3 ml normal saline, was injected intravenously, and 5 min later chemoreceptor chemosensitivity was measured again. This sequence was repeated another 30 min later after intravenous injection of 0.5 mg vecuronium. Recovery of the chemoreceptor function was then assessed by means of repeating the previous procedure at 30, 60, and 90 min after the last injection.
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Table 1. The Variation in Pa\text{CO}_2 during the Experimental Period in Each of the Seven Animals

<table>
<thead>
<tr>
<th>Rabbit Number</th>
<th>Pa\text{CO}_2 (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38 ± 2</td>
</tr>
<tr>
<td>2</td>
<td>37 ± 1</td>
</tr>
<tr>
<td>3</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>4</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>5</td>
<td>35 ± 2</td>
</tr>
<tr>
<td>6</td>
<td>37 ± 2</td>
</tr>
<tr>
<td>7</td>
<td>40 ± 3</td>
</tr>
</tbody>
</table>

Data are mean ± SD.

Time Course of the Neuromuscular Block

The neuromuscular blocking effect of vecuronium was recorded in four additional rabbits by using the same anesthetic technique. During normothermia and mechanical normoventilation, the time course of neuromuscular block was recorded using mechanomyography (Myograph 2000®, Organon Teknika, Boxtel, The Nether-

lands) of lower limb extension force after supramaximal femoral nerve train-of-four (TOF) stimulation (2 Hz for 1.5 s every 11.5 s). A stable baseline was awaited during a 10- to 15-min period, whereafter the first twitch in the TOF response (T1) was calibrated to 100% and served as control response. After injection of 0.1 mg intravenous vecuronium, the time until 25% T1 twitch recovery (Dur 25%) and time until a TOF ratio (T4/T1) of 0.70 (Dur TOF 0.70) were calculated from the recording. Thirty minutes after the 0.1-mg dose, 0.5 mg intravenous vecuronium was given, and the same time course parameters were calculated again, i.e., the time course of neuromuscular block was recorded during identical experimental conditions as in the chemoreceptor test animals.

After completion of the experiment, all animals were given a lethal dose of thiopental and potassium chloride intravenously.

Data are presented as mean ± SD and/or range. Statis-

Fig. 1. Original recording of the activity of a single chemoreceptor with the chemoreceptor output (Hz) and corresponding tracheal O\textsubscript{2} and CO\textsubscript{2} concentrations (%) and mean arterial blood pressures (mmHg). Each panel represents one of the six test occasions, i.e., control recording, after administration of 0.1 and 0.5 mg vecuronium and then during the 90-min recovery period.

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tistical analyses of the chemosensitivity index (the b coefficient) were made by comparing control measurements with the two dose administrations, i.e., values for the b coefficient obtained after normal saline injection (control) versus after administration of 0.1 mg and 0.5 mg of vecuronium. A second comparison was made between the 0.5-mg dose and the 30-, 60-, and 90-min recovery measurements, respectively. Analysis of variance for repeated measures design was used followed by Fischer’s exact test. A P value of less than 0.05 was considered to indicate statistical significance.

Results

All rabbits were normothermic and had a stable PaO₂ during the entire experimental period. The isocapnic PaCO₂ values for each animal are presented in table 1. All single fiber preparations fulfilled the criteria throughout the six study occasions with a maintained configuration of the chemoreceptor action potential.

In figure 1 the original output from a single chemoreceptor (chemo output, Hz) of one of the preparations is shown with simultaneous tracheal O₂ concentrations (%), CO₂ concentrations (%), and mean arterial blood pressures (mmHg) for the entire experimental period, i.e., during control and after administration of 0.1 mg and 0.5 mg intravenous vecuronium followed by the 30-, 60-, and 90-min recovery measurements. In the same animal, the resultant chemoreceptor response curve for the six occasions are presented in figure 2. As shown, when the control chemoreceptor response was measured before administration of vecuronium, a typical three- to fourfold increase in the firing frequency was recorded as the PaO₂ was reduced stepwise. After administration of 0.1 and 0.5 mg intravenous vecuronium, a significant dose-related reduction in the chemoreceptor activity was recorded during hypoxia, followed by a gradual recovery of the chemoreceptor output at 30, 60, and 90 min.

The mean chemoreceptor output with corresponding PaO₂, PaCO₂, pH, and mean arterial blood pressure (MAP) during the four levels of oxygenation and at the six test occasions in all animals are shown in table 2. The resultant mean chemoreceptor output with corresponding mean PaO₂ values for all experiments are presented in figures 3 and 4.

Comparing the chemosensitivity (b coefficient) before administration of vecuronium with both doses of vecuronium showed that the chemosensitivity was significantly reduced (P < 0.05); the 0.5-mg dose caused a greater depression than the 0.1-mg dose (0.1 mg vs. 0.5 mg, P < 0.05). The chemosensitivity remained depressed at 30 and 60 min after administration of the 0.5-mg vecuronium dose (P < 0.05). At 90 min after injection of vecuronium, chemosensitivity was not significantly different from control activity. A similar degree of hypoxia was obtained at all test occasions with stable
Table 2. The Chemoreceptor Output (Chemo Output) with Corresponding $P_{aCO_2}$, $P_{aO_2}$, $pH$, and Mean Arterial Blood Pressure (MAP) at Four Levels of Oxygenation for Each of the Six Test Occasions

<table>
<thead>
<tr>
<th>Control</th>
<th>0.1 mg Vecuronium</th>
<th>0.5 mg Vecuronium</th>
<th>90 min</th>
<th>60 min</th>
<th>30 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemo output</td>
<td>$P_{aCO_2}$ (mmHg)</td>
<td>$P_{aO_2}$ (mmHg)</td>
<td>$pH$</td>
<td>MAP (mmHg)</td>
<td>7.44</td>
<td>7.44</td>
</tr>
<tr>
<td>11.4 ± 3</td>
<td>19 ± 3</td>
<td>35 ± 4</td>
<td>35 ± 4</td>
<td>7.44 ± 0.02</td>
<td>7.43 ± 0.02</td>
<td>7.45 ± 0.02</td>
</tr>
<tr>
<td>16 ± 3</td>
<td>35 ± 4</td>
<td>35 ± 4</td>
<td>7.44 ± 0.02</td>
<td>7.43 ± 0.02</td>
<td>7.45 ± 0.02</td>
<td>7.45 ± 0.02</td>
</tr>
<tr>
<td>20 ± 3</td>
<td>35 ± 4</td>
<td>35 ± 4</td>
<td>7.44 ± 0.02</td>
<td>7.43 ± 0.02</td>
<td>7.45 ± 0.02</td>
<td>7.45 ± 0.02</td>
</tr>
<tr>
<td>30 ± 6</td>
<td>46 ± 6</td>
<td>46 ± 6</td>
<td>7.44 ± 0.02</td>
<td>7.43 ± 0.02</td>
<td>7.45 ± 0.02</td>
<td>7.45 ± 0.02</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

P$_{aCO_2}$, and all animals showed normal pH throughout the experiment. Notably, the effect of vecuronium on the chemoreceptor activity during hypoxia varied between preparations. This is shown in figure 5, where the individual chemosensitivity (the h coefficient) at each of the six test situations is presented. As shown, there were three chemoreceptor preparations with minor depression, whereas four preparations showed a marked depression of the chemosensitivity. Moreover, one preparation recovered only partially by 90 min but showed complete recovery at an additional measurement after 120 min (rabbit #7).

Neuromuscular Block

The time course of neuromuscular block in the four rabbits is presented in table 3. As shown, the duration until TOF ratio 0.70 after intravenous administration of 0.1 mg vecuronium was approximately 15 min, and complete recovery was achieved before administration of the 0.5-mg vecuronium dose. The 0.5-mg vecuronium dose resulted in a longer block, during which a TOF ratio of 0.70 was reached within 25–57 min (mean, 37 min), i.e., all animals showed partial neuromuscular block at the time interval between 30 and 60 min. In figure 6, an original mechanomyographic recording of the lower limb extension force in one animal is presented after intravenous administration of 0.1 and 0.5 mg vecuronium.

Discussion

The main findings in this study were that systemic administration of vecuronium depressed the carotid body chemoreceptor activity during hypoxia to a variable extent; the effect was dose-dependent, and the depression spontaneously recovered after approximately 90 min.

Critique of Method

In the present study we have recorded the activity of isolated single chemoreceptors in the intact animal over a range of P$_{aCO_2}$ and before and after administration of vecuronium. To describe the resultant chemoreceptor response curve, we applied a hyperbolic relationship, as previously used for studies of chemoreceptor function during hypoxia and to study the effect of various pharmacologic agents. In these studies on the stimulus-response characteristics, similar results have been obtained when the chemoreceptor output has been plotted.
against \( \text{Pa}_2 \), i.e., a hyperbolic response curve as previously defined by Ponte,7 Bisco,6,10 Hornbein,9 and Fi-done and Gonzalez.11 The advantage of using a single chemoreceptor preparation instead of a multifiber preparation or whole sinus nerve preparation is that the single fiber preparation is either present or absent, whereas multi- or whole nerve preparations show the sum effect when the quality of recording may vary over time.10 The disadvantages are the considerable technical difficulties involved in the single fiber preparation and maintenance of the signal and the short life span of a small single nerve fiber.10 The present study involved a meticulous preparation and maintenance of the chemoreceptor signal followed by repeated measurements of the chemoreceptor chemosensitivity for a prolonged period (i.e., 2–2.5 h).

We found a variable reduction in the chemoreceptor chemosensitivity. This variability in the effect of muscle relaxants on the chemosensitivity has been noted previously.5,5,5 Using an isocapnic test procedure in partially paralyzed volunteers, Eriksson and Severinghaus2 found that the depression of human hypoxic ventilatory varied
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Fig 5. The variation between individual chemosensitivity (b coefficients) for each of the six study occasion. Chemoreceptor output is expressed as (Hz) = a + b (PaO₂−1) (mmHg). As shown, there was a large variation in the effect of vecuronium on the chemosensitivity during isocapnic hypoxia. A significant depression in the mean chemosensitivity (b coefficient) was found, comparing 0.1 mg and 0.5 mg vecuronium to control (P < 0.05) and by comparing 0 min to 30 and 60 min (P < 0.05). At 90 min, the mean chemosensitivity did not differ from control measurements (not significant).

between 15–60%. Similar findings were later reported for pancuronium, atracurium, and vecuronium using a similar isocapnic test procedure in humans. Moreover, there is a great interindividual variation in normal hypoxic ventilatory response of humans and the neuromuscular blocking effect of vecuronium after single bolus doses. The latter was confirmed in this study of a limited number of rabbits to which mechanomyography was applied to describe the time course of vecuronium-induced neuromuscular block. It would have been valuable to simultaneously monitor the level of neuromuscular block, either by evoked electromyography or mechanical myography, and to correlate these recordings with the chemosensitivity found. We chose, however, to study the neuromuscular block in four additional rabbits to reduce the risk for destruction of the chemoreceptor preparation by simultaneous recording of mechanical twitch responses (i.e., movements). From these recordings it seems most likely that the 30- and 60-min recording of chemoreceptor chemosensitivity occurred at a partial neuromuscular block.

There are several factors such as body temperature, interference from anesthetic drugs, and hypotension\textsuperscript{7,12} that may influence the findings during this extended experiment. The animals were kept normothermic and at isonormocapnia during the entire experimental period. Thiopental was used for induction and maintenance of anesthesia because it does not interfere with chemoreceptor function and was given at a constant infusion rate during the experimental period.\textsuperscript{12} All animals had a stable mean arterial blood pressure of more than 55–60 mmHg during the experimental period. Our data show that the variable degree of chemoreceptor depression recovers spontaneously with time, and more importantly, it was possible to obtain almost the same responsiveness of the depressed chemoreceptors at the end of the experiment, i.e., after approximately 90 min. Whether full recovery could have been obtained faster after an anticholinesterase agent remains to be studied.

<table>
<thead>
<tr>
<th>Vechuronium Dose</th>
<th>0.1 mg</th>
<th>0.5 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dur 25% (min)</td>
<td>11.8</td>
<td>29.8</td>
</tr>
<tr>
<td>Range</td>
<td>10–14</td>
<td>20–43</td>
</tr>
<tr>
<td>Dur TOF 0.70 (min)</td>
<td>15.3</td>
<td>37.0</td>
</tr>
<tr>
<td>Range</td>
<td>12–18</td>
<td>23–57</td>
</tr>
</tbody>
</table>

The duration until recovery of the first twitch in the TOF response to 25% of initial control response (Dur 25%, min) and time until recovery to a TOF ratio (T4/T1) of 0.70 (Dur TOF 0.70, min) are presented (mean and ranges are given).

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Chemosenstivity and Muscle Relaxants

An interaction between hypoxic ventilatory control and subparalyzing doses of nondepolarizing neuromuscular blocking agents has recently been demonstrated in humans.\textsuperscript{1–3} This interaction seems to be a previously unknown property of several neuromuscular blocking agents in clinical use\textsuperscript{5} despite its different molecular structure classification (steroid-based vs. bensylisoquinolinium-
based) and varying muscarinic acetylcholine receptor affinity (pancuronium vs vecuronium). In the investigation of the underlying mechanisms involved, we have recently shown that hypoxia-induced increase in phrenic nerve activity in anesthetized rabbits can be partially blocked by small amounts of vecuronium given via the lingual artery near the carotid body chemoreceptors. In addition, the reduction in phrenic nerve activity during hypoxia was related to the dose of vecuronium given (1–10 μg of vecuronium). It is thus evident that minute doses of a nondepolarizing neuromuscular blocking agent directly affects chemoreceptor function, rather than central neuronal respiratory control. Based on the human data, recent in vivo results, and in vitro experiments, we suggest that neuromuscular blocking agents, in addition to muscular paralysis, cause depression of peripheral chemoreceptor activity during hypoxemia.

With our protocol, however, it is not possible to draw conclusions about the exact nature of this interaction. The importance of cholinergic transmission of the carotid bodies during hypoxia has been debated. In studies of neurotransmitter release from the isolated carotid body, it was noted that acetylcholine was released during moderate hypoxemia. Acetylcholine is not the only neurotransmitter involved in the chemical transmission of the carotid body. Other transmitters such as substance P and dopamine are also involved in carotid body function during hypoxia, most likely being the two primary neurotransmitters. Hence, the actual role of acetylcholine in chemosensitivity is not fully understood at present. Further, the role of cholinergic transmission of the carotid body for chemosensation has been challenged by several groups. Recently, the importance of intact cholinergic function was reestablished based on findings by Fitzgerald et al. In these in vitro experiments the authors demonstrated that the chemosensitivity of the isolated carotid body, measured as an increased neuronal activity of the whole sinus nerve during hypoxia, can be reversibly attenuated by a perfusion of a mixture of muscarinic and nicotinic acetylcholine antagonists. On the other hand, the effect of clinically used muscle relaxants on chemoreceptor function has never been investigated nor questioned in anesthetic practice.

There are several ways by which vecuronium can exert its action on nerve terminals. First, vecuronium has a high affinity for nicotinic cholinoreceptors as opposed to muscarinic receptors. The drug may also cause an acetylcholine receptor or calcium channel block, although the acetylcholine block is more likely to occur at higher concentrations of the antagonist than those seen during partial paralysis. Interestingly, calcium-specific ion-channels are present in chemoreceptor membrane and are important for normal chemosensation. Hence, there are several possible mechanisms by which vecuronium may depress the chemoreceptor or its chemical neurotransmission. Our findings stimulate further investigations to reach a comprehensive understanding of the mechanisms behind the interaction between a nondepolarizing muscle relaxant and hypoxic ventilatory control.

We conclude that vecuronium may depress chemoreceptor function to a varying extent during hypoxia and that the depression recovers spontaneously.

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References


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