EFFECT OF IONIZED CALCIUM ON THE NEUROMUSCULAR BLOCKING ACTIONS OF ATRACURIUM AND VECURONIUM IN THE CAT

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SUMMARY
We have determined neuromuscular blocking effects of atracurium and vecuronium at normal, high and low plasma concentrations of ionized calcium ([Ca^{2+}]) in the cat. Twitch responses were measured bilaterally in the anterior tibia/is muscles, using intact central innervation in one preparation. Plateaus of high and low [Ca^{2+}] were created by infusions of calcium chloride and citrate, respectively. The interactions with changes in [Ca^{2+}] were similar for atracurium and vecuronium, and were unaffected by central muscle innervation. The median increase in [Ca^{2+}] from 1.27 to 1.59 mmol litre^{-1} shifted the dose–response curves of the drugs to the right, increasing ED_{50} by 7–13%, whereas the decrease to median 0.78 mmol litre^{-1} potentiated the drugs by a similar order. This indicates a lesser influence of [Ca^{2+}] on the action of neuromuscular blockers than reported in a previous in vitro study. Even though the interactions were statistically significant, their moderate magnitude suggests minor clinical significance.

KEY WORDS

Waud and Waud measured the effect of ionized calcium on block produced by tubocurarine or pancuronium in vitro, and estimated a 27% variation in dose requirement over a physiological range of concentrations of Ca^{2+} (1.06–1.31 mmol litre^{-1}) [1].

In 30 uraemic patients [2], the serum concentration of ionized calcium ([Ca^{2+}]) was in the range 0.77–1.61 mmol litre^{-1}. In these patients we found no association between the responses of neuromuscular blocking agents and [Ca^{2+}] (unpublished data). To elucidate this apparent discrepancy between the in vitro and the clinical observations, we have studied the interaction between ionized calcium and atracurium or vecuronium in the in vivo animal model. Furthermore, we tested if calcium effects could be modified via central innervation to the muscle.

MATERIAL AND METHODS
We used 14 cats, bred for biomedical research at our animal department, of both sexes, age 13.5–21 months and weights between 2.8–5.4 kg.

Anaesthesia and surgical preparation
Anaesthesia was induced with pentobarbitone 20–30 mg kg^{-1} i.p. with supplementary i.v. increments as required, and maintained with an i.v. infusion of the barbiturate at a rate of 4–7 mg kg^{-1} h^{-1} using a 0.5% solution in 3.5% glucose with 0.3% sodium chloride. Following tracheal intubation, mechanical ventilation was commenced at a rate of 27 b.p.m. with 40% oxygen in air. The experiment was conducted in stages with control of pH and temperature. Tidal volume was adjusted to maintain pH at 7.30–7.45. Arterial blood-gas tensions and pH were monitored regularly (Radiometer ABL1). A circulating water blanket was used to maintain rectal temperature at 37.5–39.5 °C (Digimed H10 thermometer). A carotid artery was cannulated for blood sampling and arterial pressure monitoring. The ECG was recorded. An external jugular vein was cannulated for infusion of 3.5% glucose with 0.3% sodium...
chloride, containing potassium chloride 4 mmol litre^{-1}. When the surgical preparation was completed, this solution also included sodium dihydrogen phosphate 10 mmol litre^{-1}. A third vein was cannulated for infusion of the neuromuscular blocking drug. Total fluid load was 7–9 ml kg^{-1} h^{-1}.

The tendon of each anterior tibialis muscle was freed and connected to a Statham UC3 transducer equipped with a UL4-10 load cell. The output of the transducers was registered on a Graphite Linearacorder WR3101 or a Watanabe Linearacorder WTR331 using Hewlett-Packard 8805C amplifiers. The resting tension of the muscles was held at 45–50 g. Digimed F3 temperature probes were placed subcutaneously on the muscle bellies (unilateral measurements in four cats). A drape of plastic foil (aided occasionally by the radiation of an electric lamp) was used when required to keep muscle surface temperatures at 34.5–37 °C.

The tibial nerves were freed from the peroneal nerves and sectioned near the popliteal space and proximally on the thigh. The peroneal nerve on one side was sectioned likewise proximally, whereas this nerve was saved in the other limb. A shielded bipolar platinum electrode was placed on each peroneal nerve, and supramaximal stimuli were applied at 0.1 Hz (Myotest MKII nerve stimulators). The hind limbs were fixed securely to a rigid frame by pointed clamps at the knees and at the ankles. Wound edges were sutured.

**Drugs**

Atracurium and vecuronium were diluted in 0.9 % saline to concentrations of 0.3 mg ml^{-1} and 0.12 mg ml^{-1}, respectively. Calcium chloride 1 mmol ml^{-1} was diluted in 5 % glucose to a concentration of 0.05 mmol ml^{-1}. A formulation of sodium citrate 230 mg ml^{-1} and citric acid 15 mg ml^{-1} was diluted with sterile water to a concentration of 0.27 mmol ml^{-1}.

**Procedure**

Seven cats received vecuronium and a consecutive group of seven animals received atracurium.

Figure 1 shows the experimental course. The neuromuscular blockers were administrated by continuous infusion, which was titrated to obtain constant infusion–response conditions at consecutive 25%, 50%, 75% and 50% blocks, determined from the muscle without a central innervation. After at least 1 h infusion time at each response level, steady-state conditions were assumed, and the drug requirement and the twitch response were noted. After a subsequent 15-min period of constant-rate infusion the twitch response was re-assessed. While the infusion of the neuromuscular blockers was maintained stable, calcium chloride or citrate was initially infused at a fast constant rate followed by a slow constant-rate infusion to create plateaus of high or low [Ca^{2+}], respectively. The calcium chloride infusion rate (mmol kg^{-1} h^{-1}) was 3.6 for 1 min, followed by 0.25 for 6 min. The citrate infusion rate (mmol kg^{-1} h^{-1}) was 4.0 for 3 min followed by 0.8 for 6 min. This mode of administration was intended to increase or decrease [Ca^{2+}] by about 0.4 mmol litre^{-1}. If [Ca^{2+}] had not returned to normal 1 h after previous calcium chloride or citrate administration, the infusion of the neuromuscular blocker was prolonged accordingly. [Ca^{2+}] was measured (ICA1 Ionized Calcium Analyzer, Radiometer, Denmark) at each end of a 3-min time interval at the end of the infusion of calcium chloride or citrate, during which period the median change in neuromuscular block was determined.

Initially, during control twitch height we gave calcium chloride to the vecuronium group and citrate to the atracurium group. In all animals calcium chloride was given during the subsequent

![Fig. 1. The experimental stages. Consecutive levels of neuromuscular blocks were produced by continuous infusions of stracurium or vecuronium. The broken lines indicate the approximate steady-state periods, when [Ca^{2+}] was altered by brief infusions of calcium chloride (Ca) or citrate (Cit).](https://academic.oup.com/bja/article-abstract/64/2/199/260168)
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25 %, 50 % and 75 % blocks, and citrate was given during the final 50 % block.

Statistics
The Wilcoxon signed mid-rank test was used for analysis of changes within groups. Infusion rate–response relationships were determined by a variance component analysis of individual regressions, using infusion rate as the dependent variable. Differences were considered statistically significant if \( P < 0.05 \).

RESULTS
Age and weight in the two groups of cats were (median and total range): atracurium 18.5 months (13.5–21 months) and 4.0 kg (2.8–4.7 kg), vecuronium 18 months (18–18.5 months) and 3.9 kg (3.0–5.4 kg).

The distribution of blocks at the constant infusion–response intervals are shown in figure 2. Even though twitch-to-twitch variation was more pronounced in the muscles with preserved central reflexes, the overall stability of individual responses was similar in both muscles in both drug groups. The median individual changes in twitch height from start to end of these four 15-min intervals (in % of control in absolute values, neglecting the direction of the changes) were: vecuronium group 1.7 % and atracurium group 0.8 %.

During the last 3 min of the slow infusion sequence of calcium chloride or citrate, the plateaus of increased or decreased \([\text{Ca}^{2+}]\) were relatively stable and close to the intended concentrations of about 1.6 and 0.8 mmol litre\(^{-1}\), respectively (fig. 3). The overall median concentrations during low, normal and high \([\text{Ca}^{2+}]\) were 0.78, 1.21 and 1.59 mmol litre\(^{-1}\), respectively. From start to end of the 3-min measurement intervals of high or low \([\text{Ca}^{2+}]\), the median individual changes in \([\text{Ca}^{2+}]\) (absolute values) were 0.02 and 0.01 mmol litre\(^{-1}\), respectively, and were similar for both drug groups. There were no major differences in \([\text{Ca}^{2+}]\) between the comparative test periods or the two drug groups (table I). pH and temperatures were comparable in the various test periods and the two groups (table I).

In the non-curarized animals, the twitch force was attenuated rapidly when \([\text{Ca}^{2+}]\) increased.
(table II), whereas the twitch force was augmented rapidly when \([\text{Ca}^{2+}]\) was decreased (table III). The twitch response was relatively stable during the 3-min period when the control values for the altered \([\text{Ca}^{2+}]\) values were determined. The median individual changes (as % of the control value) at normal \([\text{Ca}^{2+}]\) from start to end of this 3-min time interval were 0.4% (real value: i.e. taking the direction of the change into account) and 0.4% (absolute value) for both muscles when \([\text{Ca}^{2+}]\) was increased. When \([\text{Ca}^{2+}]\) was decreased in the non-curarized animal, the corresponding values were 0.4%, 1.0% (centrally denervated muscle) and 0%, 1.3% (centrally innervated muscle).

The increase in \([\text{Ca}^{2+}]\) enhanced the twitch

![Graph showing concentrations of ionized calcium in plasma before and during the infusions of calcium chloride or citrate at the various levels of neuromuscular block. Medians and total ranges are presented for the two groups of cats: ○ = atracurium; □ = vecuronium.](image_url)
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TABLE II. Twitch depressions in the centrally denervated and innervated anterior tibialis muscles at the various response levels during normal and high [Ca 2+]. The quoted responses are adjusted to the decrease in control twitch height at increased [Ca 2+]. Values are medians and total ranges. The increase in [Ca 2+] produced significant changes in twitch height in all cases (P < 0.05).

<table>
<thead>
<tr>
<th>Drug and central innervation of muscle</th>
<th>Control</th>
<th>25% Block</th>
<th>50% Block</th>
<th>75% Block</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (%)</td>
<td>High (%)</td>
<td>Normal (%)</td>
<td>High (%)</td>
</tr>
<tr>
<td>Atracurium Denervated</td>
<td>0 — 25.3 (21.8-28.5)</td>
<td>0.5 (-2.7-11.1)</td>
<td>50.0 (46.3-53.0)</td>
<td>33.0 (24.2-42.6)</td>
</tr>
<tr>
<td>Innervated</td>
<td>0 — 20.4 (6.3-27.2)</td>
<td>4.9 (-6.7-14.9)</td>
<td>47.6 (30.5-53.5)</td>
<td>24.8 (15.3-45.2)</td>
</tr>
<tr>
<td>Vecuronium Denervated</td>
<td>0 5.4 (4.4-7.0)</td>
<td>23.8 (21.5-27.9)</td>
<td>11.8 (5.3-14.0)</td>
<td>49.8 (47.1-50.8)</td>
</tr>
<tr>
<td>Innervated</td>
<td>0 5.5 (4.6-6.8)</td>
<td>23.3 (13.3-40.2)</td>
<td>13.5 (3.1-30.0)</td>
<td>50.0 (42.1-59.5)</td>
</tr>
</tbody>
</table>

TABLE III. Twitch depressions in the centrally denervated and innervated anterior tibialis muscles at control and 50% twitch response during normal and low [Ca 2+]. The quoted responses during neuromuscular block are adjusted to the increase in control twitch height at decreased [Ca 2+]. Values are medians and total ranges. The decrease in [Ca 2+] caused significant changes in twitch height (P < 0.05) in all groups, except during 50% block for the centrally denervated muscle in the atracurium group (P = 0.052).

<table>
<thead>
<tr>
<th>Drug and central innervation of muscle</th>
<th>Control</th>
<th>50% Block</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (%)</td>
<td>Low (%)</td>
</tr>
<tr>
<td>Atracurium Denervated</td>
<td>0 — -6.6 (-12.4 to -4.8)</td>
<td>49.0 (44.8-57.5)</td>
</tr>
<tr>
<td>Innervated</td>
<td>0 — -7.2 (-19.0 to -1.8)</td>
<td>49.6 (26.3-56.2)</td>
</tr>
<tr>
<td>Vecuronium Denervated</td>
<td>0 — 48.1 (42.9-52.1)</td>
<td>89.5 (62.5-96.5)</td>
</tr>
<tr>
<td>Innervated</td>
<td>0 — 52.0 (43.3-63.3)</td>
<td>72.8 (39.9-93.8)</td>
</tr>
</tbody>
</table>

height during the three levels of atracurium or vecuronium induced blocks (table II), whereas decreasing [Ca 2+] potentiated the neuromuscular blocks of the two drugs (table III). The interactions developed rapidly to relatively stable response plateaus. When considering each of the four test periods for each innervation status and drug, the median individual change during the 3-min measurement intervals was -0.9 to 1.9% (real values) or 0-2.1% (absolute values) of control twitch height. Recordings of the effects of high and low [Ca 2+] are shown in figure 4.

Infusion rate–response curves are presented in figure 5. Regression-derived infusion rate–response values are summarized in table IV.

DISCUSSION

Acute increase in [Ca 2+] from normal to about 1.6 mmol litre⁻¹ antagonized the neuromuscular blocking effects of atracurium and vecuronium similarly in the cat. The acute reduction in the concentration of Ca 2+ from normal to about 0.8 mmol litre⁻¹ potentiated both drugs. There were no major differences in the median calcium effects whether the muscle was centrally innervated or not.
Fig. 4. Recordings showing the effects of high and low [Ca\textsuperscript{2+}] on the twitches of the centrally denervated anterior tibialis muscle. Upper panels: responses before the administration of neuromuscular blocking drugs; lower panels: during 50\% neuromuscular block. Smaller transient elevations of base line during the citrate infusion were observed in two other cats. The selected recordings represent the median change in twitch height which was closest to the mean values in the 14 cats.

Alteration of [Ca\textsuperscript{2+}]

In addition to the effects on [Ca\textsuperscript{2+}], infusions of calcium chloride or citrate may also interact with plasma magnesium. The active ionized fraction of magnesium in human plasma is 55\%, and the inactive remainder is either protein-bound (32\%) or chelated as low molecular weight complexes (13\%) [3]. For calcium the corresponding approximate values are 45–50\%, 40\% and 10–12\%, respectively [4].

Pedersen estimated the relationship for the equilibrium between the serum protein binding and the ionic concentrations of these two competitive divalent cations [5]. According to his formulae, the increase of [Ca\textsuperscript{2+}] from 1.2 to 1.6 mmol litre\textsuperscript{-1} makes the protein-binding of magnesium about 1 percentage point less, which is negligible. The effect of Ca\textsuperscript{2+} on low molecular weight magnesium complexes is unknown.

Infused citrate forms complexes with calcium in addition to magnesium. Because the dissociation constant of calcium citrate and magnesium citrate is the same ($10^{-9.22}$) [6], citrate reduces the concentrations of Mg\textsuperscript{2+} and Ca\textsuperscript{2+} in the same proportion, provided that there is negligible interaction from other complexing agents.

Fig. 5. The median dose-response relationships for vecuronium (■ ■) and atracurium (○ ○) are demonstrated for the three values of [Ca\textsuperscript{2+}]. Open symbols represent the muscle preparations with intact central innervation. The dose-response curves at normal [Ca\textsuperscript{2+}] (solid lines) were shifted similarly to the right (broken lines) when [Ca\textsuperscript{2+}] was increased by about 0.4 mmol litre\textsuperscript{-1}. When [Ca\textsuperscript{2+}] was decreased by about 0.4 mmol litre\textsuperscript{-1} during 50\% response, the depression of twitch height was potentiated (single symbols).
TABLE IV. Dose–response regression data for atracurium and vecuronium during normal and high [Ca\(^{2+}\)], using anterior tibialis muscles without or with a central innervation. Values are mean with 95% confidence intervals

<table>
<thead>
<tr>
<th>Drug and [Ca(^{2+})]</th>
<th>Denervated</th>
<th>Innervated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED(_{50}) ((\mu g) kg(^{-1}) h(^{-1}))</td>
<td>Slope (log)</td>
</tr>
<tr>
<td>Atracurium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal [Ca(^{2+})]</td>
<td>226 (204–251)</td>
<td>0.42 (0.20–0.64)</td>
</tr>
<tr>
<td>High [Ca(^{2+})]</td>
<td>255 (228–290)</td>
<td>0.36 (0.18–0.53)</td>
</tr>
<tr>
<td>Vecuronium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal [Ca(^{2+})]</td>
<td>101 (93–110)</td>
<td>0.29 (0.11–0.47)</td>
</tr>
<tr>
<td>High [Ca(^{2+})]</td>
<td>111 (100–123)</td>
<td>0.31 (0.13–0.48)</td>
</tr>
</tbody>
</table>

To avoid hypophosphataemia from moderate administration of glucose during the prolonged experimental course, phosphate was added to the infusion fluid [7]. Low concentrations of phosphate in plasma may impair neuromuscular function and hamper the restoration of increased [Ca\(^{2+}\)] to normal [8].

**Neuromuscular responses**

Effects of varying calcium concentration on atracurium- or vecuronium-induced blocks have not been reported previously. If we assume approximated steady-state conditions during our test periods, the rate of infusion relates directly to drug concentration for the given response. Using an isolated guineapig nerve–lumbrical muscle preparation, Waud and Waud found that the actions of pancuronium and tubocurarine were affected similarly by changes in concentration of calcium [1]. This compares to our finding that calcium interacted similarly with atracurium and vecuronium.

The size of interaction was, however, less in our study than that reported by Waud and Waud. The median change of [Ca\(^{2+}\)] from 1.21 to 1.59 mmol litre\(^{-1}\) increased ED\(_{50}\) by 7–13% in our in vitro preparations, whereas it can be calculated from the in vitro data of the former workers that a similar increase in [Ca\(^{2+}\)] would lead to a 35% increase in ED\(_{50}\) [1].

When [Ca\(^{2+}\)] was decreased to median 0.78 mmol litre\(^{-1}\), the potentiating of atracurium and vecuronium was of similar magnitude as the antagonism observed at high [Ca\(^{2+}\)]. As discussed above, the decrease in [Ca\(^{2+}\)] produced by citrate is accompanied by a proportional reduction in plasma concentration of ionized magnesium ([Mg\(^{2+}\)]. To our knowledge, it has not been reported how the action of neuromuscular blocking drugs is affected by hypomagnesaemia. Because a reduction in [Mg\(^{2+}\)] markedly increases the amount of evoked acetylcholine released from the motor nerve terminal [9], it is likely that these non-depolarizing agents are antagonized by low [Mg\(^{2+}\)]. Accordingly, we have probably underestimated the interaction with atracurium and vecuronium caused by low [Ca\(^{2+}\)]. However, the pronounced depression of twitch responses associated with the citrate infusion shows that a decrease in [Mg\(^{2+}\)] has considerably less effect on the neuromuscular blocking action of atracurium and vecuronium than a proportional decrease in [Ca\(^{2+}\)].

Ca\(^{2+}\) interactions were similar in the centrally innervated and denervated limb. Therefore, the disagreement between our clinical and the reported in vitro findings cannot be explained by centrally mediated neural activity masking the direct action of Ca\(^{2+}\) at the neuromuscular junction.

Even though acute changes in [Ca\(^{2+}\)] produced statistically significant interactions with atracurium and vecuronium, the magnitude of these effects might be considered clinically insignificant. This would agree with the lack of association between [Ca\(^{2+}\)] and the potency of neuromuscular blockers in renal failure patients. Such patients have large inter-individual differences in their
sensitivity to neuromuscular blocking drugs [2], which confounds moderate interactions of Ca\(^{2+}\). The normal physiological range of [Ca\(^{2+}\)] in other patients probably affects the drug responses negligibly.

ACKNOWLEDGEMENT

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REFERENCES