

Betamethasone-Induced Resistance to Neuromuscular Blockade: A Comparison of Atracurium and Vecuronium In Vitro

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Steroids induce resistance to neuromuscular blocking drugs. Betamethasone-induced resistance to vecuronium has been demonstrated in vitro, and a presynaptic site of interaction has been suggested. This study investigated whether atracurium is similarly affected. Rat phrenic nerve-hemidiaphragm preparations were bathed in a physiologic solution, and one-half were exposed to betamethasone ($1 \mu\text{mol/L}$). Dose responses were recorded for atracurium ($8\text{--}13 \mu\text{mol/L}$) and vecuronium ($2\text{--}12 \mu\text{mol/L}$) for control and betamethasone-treated preparations. In comparison to control, the betamethasone groups had significantly less depression of muscle contraction force at all concentrations of atracurium ($P = 0.0004$) and vecuronium ($P = 0.002$). The calculated ED_{50} (50%

depression of muscle contraction force, expressed as mean \pm SEM) for atracurium was $8.83 \pm 0.62 \mu\text{mol/L}$ for controls and $11.19 \pm 0.54 \mu\text{mol/L}$ for betamethasone-treated preparations. The calculated ED_{50} for vecuronium was $4.72 \pm 0.41 \mu\text{mol/L}$ for controls and $6.84 \pm 0.66 \mu\text{mol/L}$ for betamethasone-treated preparations. Betamethasone therefore increased the ED_{50} for atracurium by 27% and vecuronium by 45%; however, the magnitudes of these differences were not significant ($P = 0.74$) between the neuromuscular blocking agents. These results indicate that betamethasone-induced resistance to nondepolarizing neuromuscular blockade affects both atracurium and vecuronium to similar degrees in vitro.

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Steroids have a facilitatory effect on neuromuscular function; steroid-induced resistance to competitive nondepolarizing neuromuscular blockade has been reported (1-3). Recently we described two neurosurgical patients pretreated with betamethasone who had an apparent resistance to the effects of vecuronium and, in a subsequent review of anesthesia case records, it was found that patients pretreated with betamethasone required, on average, 75% more vecuronium than patients not pretreated (4). An interaction between betamethasone and vecuronium has since been demonstrated in vitro using rat phrenic nerve-hemidiaphragm preparations. One mechanism likely for this interaction is facilitated acetylcholine release owing to the effect of betamethasone on the presynaptic motor nerve terminal (5).

Vecuronium- and atracurium-induced neuromuscular blockades could occur through different receptor-sites; vecuronium has been suggested to act predominantly at presynaptic receptor sites (6,7), whereas atracurium has been suggested to act principally at postsynaptic receptor sites (8). In view of a possible presynaptic facilitatory effect of steroids on neuromuscular transmission, atracurium neuromuscular blockade may be less affected by betamethasone than vecuronium neuromuscular blockade. The aim of this study was to compare the effect of betamethasone on neuromuscular blockade with either vecuronium or atracurium using the in vitro rat hemidiaphragm preparation.

Methods

This study was approved by our Animal Ethics Committee. Male Dark Agouti rats (8-10 wk) weighing 150-200 g were killed by cervical dislocation. The right and left hemidiaphragms with accompanying phrenic nerves were dissected out, placed horizontally in individual organ baths (Res-Del Group, Well-

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tion may have clinical implications. Resistance to pancuronium and vecuronium given in association with steroid therapy has been reported in several cases, and this may be indicated by unexpected patient movement (1-4). Animal studies have demonstrated resistance to the effects of *d*-tubocurarine in the presence of prednisolone, dexamethasone, betamethasone, and triamcinolone (10-14). Recently we demonstrated that the reduction in twitch tension caused by vecuronium, is considerably attenuated in the presence of 1 $\mu\text{mol/L}$ betamethasone (5). Previous experimental evidence suggests that facilitatory actions of corticosteroids at the presynaptic terminal of the neuromuscular junction reduce competitive neuromuscular blockade as a consequence of steroid-enhanced acetylcholine synthesis and release (15-21). There is now considerable evidence suggesting that the significant mode of action of many clinically used nondepolarizing neuromuscular blocking drugs is due to binding at presynaptic receptors (22,23). A presynaptic cholinergic receptor binding interaction between steroids and neuromuscular blocking agents that have steroid-derived molecular structures (e.g., pancuronium, vecuronium) is one possible explanation for the apparent resistance to neuromuscular blockade (4,5). It would therefore be of clinical interest to know if a similar interaction would occur between steroids and nondepolarizing neuromuscular blocking agents that act postsynaptically or that do not have steroid-derived molecular structures. Atracurium could be one such drug; it has a chemical structure dissimilar to steroid molecular structures (i.e., betamethasone and vecuronium) and electrophysiologic investigations have suggested that atracurium acts primarily by postjunctional receptor blockade (8). In addition, synergy of vecuronium and atracurium used in combination has been reported; this has been suggested to indicate that vecuronium and atracurium act at different receptor sites that may be predominantly presynaptic and postsynaptic, respectively (24,25).

The investigations reported here indicate that, *in vitro*, atracurium neuromuscular blockade can be antagonized by betamethasone and the magnitude of this antagonism (i.e., the increase in the ED_{50}) is not statistically different from the magnitude of betamethasone-induced antagonism of vecuronium neuromuscular blockade. The similarity of the effect of betamethasone on atracurium and vecuronium that both atracurium and vecuronium act presynaptically (22). This suggests that, clinically, atracurium may not provide greater protection than vecuronium from betamethasone antagonism of neuromuscular blockade.

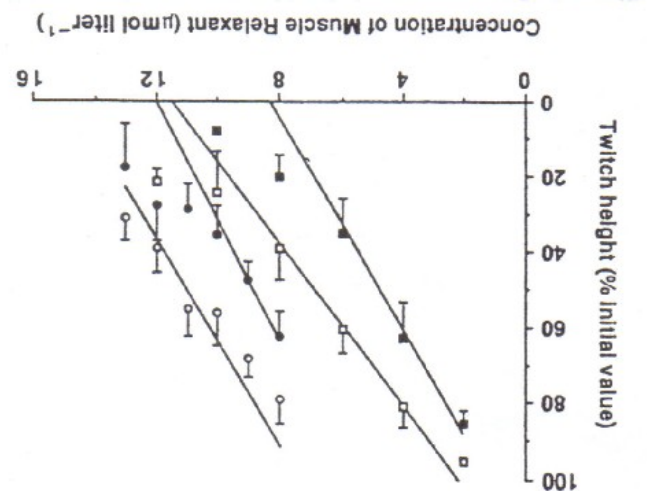


Figure 1. Dose-response relationships for atracurium control (closed circles), atracurium betamethasone-pretreated (open squares), and vecuronium betamethasone-pretreated (open circles). Data are shown as mean \pm SEM with related dose-response curves.

Table 1. Atracurium and Vecuronium Dose-Response Data for Control and Betamethasone-Treated Preparations*

Muscle relaxant	Concentration ($\mu\text{mol/L}$)	Control		Betamethasone	
		n	Response %	n	Response %
Atracurium	8	8	62.5 \pm 6.8	7	79.1 \pm 6.3
	9	8	47.5 \pm 5.3	7	68.3 \pm 5.1
	10	7	35.6 \pm 8.0	8	55.9 \pm 9.0
	11	7	29.0 \pm 7.3	7	55.0 \pm 7.3
	12	5	27.6 \pm 9.4	6	38.8 \pm 6.5
	13	2	17.5 \pm 11.5	5	31.0 \pm 6.2
	2	8	85.3 \pm 3.5	6	95.7 \pm 0.3
	4	8	63.0 \pm 9.8	8	81.0 \pm 5.3
	6	8	35.0 \pm 9.1	8	60.5 \pm 6.4
	8	2	20.0 \pm 6.0	8	38.8 \pm 8.4
	10	1	8.0	3	23.3 \pm 11.1
	12	0	—	2	21.5 \pm 3.5

*Values are mean \pm SEM. Responses are expressed as the percentage of the muscle contraction 20 min after administration of the muscle relaxant compared to the muscle contraction before each dose.

treated preparations. The calculated ED_{50} for vecuronium was $4.72 \pm 0.41 \mu\text{mol/L}$ for controls and $6.84 \pm 0.66 \mu\text{mol/L}$ for betamethasone-treated preparations. Betamethasone therefore increased the ED_{50} for atracurium by 27% and vecuronium by 45%; however, the magnitude of these differences was not significant ($P = 0.74$) between vecuronium and atracurium.

Discussion

Several case reports suggest that an interaction between corticosteroids and the neuromuscular junc-

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rectly evoked contractions. Relaxant-free perfusate was used to wash the preparations between doses to obtain indirectly evoked muscle contractions within 1.0 g of the baseline. The muscle was stretched to a basal preload tension of 4.0 g before exposure to subsequent doses of muscle relaxant.

To ensure that preparations had not deteriorated during the experimental period, data were not included in the analysis if the response to direct stimulation had decreased by more than 2.0 g at the end of the experiment.

Data from the four groups were compared using analysis of covariance, with the dose of relaxant as the covariate and terms for control or betamethasone groups, atracurium or vecuronium, an interaction term, and a term for the individual rat within atracurium and vecuronium groups. Tests for differences in slopes were made by testing dose interaction terms in the model. Dose-response relationships were calculated from responses that were less than 100% and greater than 0% of the initial twitch height. The standard errors for the ED_{50} were calculated from the ED_{50} of the individual hemidiaphragm preparation.

Results

Data were obtained from 32 hemidiaphragm preparations from 16 rats (paired preparations of vecuronium/control and vecuronium/betamethasone; paired preparations of atracurium/control and atracurium/betamethasone; eight hemidiaphragm preparations in each group). The baseline muscle contraction forces (mean \pm SEM) for atracurium/control and for atracurium/betamethasone-treated preparations were 25.4 ± 5.6 g and 22.6 ± 6.4 g, respectively. The baseline muscle contraction forces for vecuronium/control and for vecuronium/betamethasone-treated preparations were 24.1 ± 4.9 g and 23.1 ± 6.9 g, respectively. There were no changes of the direct or indirect muscle contraction forces after administration of betamethasone. The calculated ED_{50} for vecuronium was 4.72 ± 0.41 μ mol/L for controls and 6.84 ± 0.66 μ mol/L for betamethasone-treated preparations. The dose-response relationships and raw data are shown in Figure 1 and Table 1, respectively. The slopes of the dose-response lines in control and betamethasone-treated preparations were not significantly different for vecuronium ($P = 0.09$) or for atracurium ($P = 0.67$). When common slopes were fitted, in comparison to control, the betamethasone groups had significantly less depression of muscle contraction force at all concentrations of atracurium ($P = 0.0004$) and vecuronium ($P = 0.002$). The calculated ED_{50} (50% depression of muscle contraction force, expressed as mean \pm SEM) for atracurium was 8.83 ± 0.62 μ mol/L for controls and 11.19 ± 0.54 μ mol/L for betamethasone-

ington, New Zealand), and independently perfused at 2 mL/min with a physiologic solution (RS-1 Mammalian Solution, ICP, Auckland, New Zealand) aerated with 5% CO_2 and 95% O_2 (9). The solution composition and concentration of the physiologic solution (in mmol/L) was Na^+ 136.0, Cl^- 118.0, K^+ 5.0, Ca^{2+} 1.2, Mg^{2+} 0.45, HCO_3^- 25.0, N,N -bis [2-hydroxyethyl]-2-aminoethanesulfonic acid (BES) 5.0, p -glucose 10.0, glycerol 0.11, L -aspartate 0.02, L -glutamate 0.30, L -glutamine 0.40, D,L -carnitine 0.05, choline 0.01, cocarboxylase (TRP) 0.043, and insulin (porcine) 25 mIU/L.

The temperature of the perfusate was maintained at $34.5 \pm 0.5^\circ C$ using a Res-Del Temperature Control Unit, and the muscle was stretched to a basal preload tension of 4.0 g. Each preparation was attached to an isometric force transducer with a steel hook inserted through the central tendon of the hemidiaphragm. Preparations were stimulated indirectly using suction electrodes attached to the phrenic nerve, with continuous biphasic double square-wave pulses (12-ms pulse interval) at supramaximal voltages of 0.1-ms duration at 0.2 Hz using a direct current neurophysiologic stimulator of our own design. Direct stimulation (supramaximal monophasic pulses at 5 Hz) of short duration (30 s) using steel needle electrodes was also performed to examine any local action of betamethasone or vecuronium on the muscle or deterioration of the preparations. Contractions were recorded isometrically and displayed on a pen chart recorder.

At least 30 min was allowed to achieve ionic and metabolic equilibrium of the isolated preparations and a steady state of muscle contractility. Muscle contraction (twitch) force to indirect and direct stimulation of preparations in the absence of steroid or muscle relaxant was measured and recorded. One of the hemidiaphragm preparations from each rat was exposed to 1 μ mol/L betamethasone (Glaxo, Palmerston North, New Zealand) for the duration of the investigation. Equal numbers of left and right hemidiaphragms were treated with betamethasone. Control and betamethasone-treated preparations were exposed in increasing concentration to vecuronium (2, 4, 6, 8, 10, and 12 μ mol/L) or atracurium (8, 9, 10, 11, 12, and 13 μ mol/L). Muscle relaxants were administered directly to the perfusion baths (of known volume) using precision microliter syringes. Stock solutions were prepared from the commercially available preparations (vecuronium: Organon Teknika, Sydney, Australia; atracurium: Wellcome, Beckenham, United Kingdom) and stored at $4^\circ C$ before and during the experiments. Muscle contraction force was continuously recorded. The depression of muscle contraction force was calculated as the percentage of the indirectly evoked contraction 20 min after administration of the muscle relaxant to the predose indi-