INTERACTION BETWEEN BETAMETHASONE AND VECURONIUM

S. M. PARR, B. J. ROBINSON, D. REES AND D. C. GALLETLY

SUMMARY

A possible interaction between betamethasone and vecuronium was examined in 20 rat phrenic nerve–hemidiaphragm preparations. Ten preparations were bathed in a physiological solution with betamethasone 1 $\mu mol\ litre^{-1}$ added and, after a 30-min period were exposed to vecuronium at concentrations of 4, 6, 8 and 10 $\mu mol\ litre^{-1}$ with vecuronium free washings between each exposure. Ten control experiments were performed also using a betamethasone-free bathing solution. In comparison with control, the betamethasone group had significantly (P = 0.0008) less depression of muscle contraction (twitch) force at all concentrations of vecuronium. The calculated ED_{50} (50% depression of muscle contraction force) was 5.65 $\mu mol\ litre^{-1}$ for controls and 7.39 $\mu mol\ litre^{-1}$ for betamethasone-pretreated preparations. This study confirms our previous clinical observations that an interaction occurs between vecuronium and betamethasone which is characterized by resistance to neuromuscular block.

Steroids have been known for several years to facilitate neuromuscular function, and steroid induced resistance to competitive neuromuscular blocking drugs has been noted in three previous case reports [1–3]. Recently we reported two neurosurgical patients treated with betamethasone who had apparent resistance to vecuronium, and in a subsequent review of anaesthesia case records we found that patients treated with betamethasone required on average, 75% more vecuronium than patients not treated [4]. In this study we have attempted to demonstrate an interaction between betamethasone and vecuronium in a rat phrenic nerve–hemidiaphragm preparation.

MATERIALS AND METHODS

Male Dark Agouti rats (8–10 weeks) weighing 150–200 g were killed by cervical dislocation. The right and left hemidiaphragms with accompanying phrenic nerves were dissected out, placed horizontal in individual Res-Del organ baths and bathed with a physiological solution (RS-1 mammalian solution) aerated with 5% carbon dioxide in oxygen [5]. The composition of the solution is summarized in table I.

The bathing temperature was maintained at 34.5 ± 0.5 °C and the muscle stretched to a basal preload tension of 4 g. Each preparation was attached to an isometric force transducer inserted through the central tendon of the hemidiaphragm and was stimulated indirectly using suction electrodes attached to the phrenic nerve, with a continuous biphasic double pulse (12-ms pulse interval) square wave at a supramaximal voltage of 0.1 ms duration at 0.2 Hz. Short periods (30 s) of direct muscle stimulation (supramaximal monophasic stimulation at 5 Hz) were performed also using muscle electrodes to exclude any local action of betamethasone or vecuronium on the muscle. Contractions were recorded isometrically and displayed on a pen chart recorder.

S. M. PARR, B.M., F.C.ANAES.; B. J. ROBINSON, B.SC., M.SC.; D. C. GALLETLY, F.F.A.R.A.C.S., F.C.ANAES.; Section of Anaesthesia, Wellington School of Medicine, P.O. Box 7343, Wellington South, New Zealand; D. REES, B.SC., PH.D., School of Biological Sciences, Victoria University, Wellington, New Zealand. Accepted for Publication: March 21, 1991.
Correspondence to D. C. G.

TABLE I. *Solute composition and concentration of the physiological solution.* $BES = N,N\text{-}bis[2\text{-}$
hydroxyethyl]-2-aminoethanesulphonic acid

Na^+ 136.0 mmol litre^{-1}	Glycerol 0.11 mmol litre^{-1}
Cl^- 118.0 mmol litre^{-1}	L-Aspartate 0.02 mmol litre^{-1}
K^+ 5.0 mmol litre^{-1}	L-Glutamate 0.30 mmol litre^{-1}
Ca^{2+} 1.2 mmol litre^{-1}	L-Glutamine 0.40 mmol litre^{-1}
Mg^{2+} 0.45 mmol litre^{-1}	DL-Carnitine 0.05 mmol litre^{-1}
HCO_3^- 25.0 mmol litre^{-1}	Choline 0.01 mmol litre^{-1}
BES 5.0 mmol litre^{-1}	Cocarboxylase (TTP) 0.043 mmol litre^{-1}
D-Glucose 10.0 mmol litre^{-1}	Insulin (porcine) 25 miu litre^{-1}

Where possible, the preparations were used in pairs from the same rat, in independently perfused double organ baths with equal numbers of left/right hemidiaphragms used for each group. The flow rate of the perfusate was 2 ml min^{-1}. At least 30 min was allowed for a steady state to be achieved; muscle contraction (twitch) force measurements produced by indirect and direct stimulation of preparations in the absence of steroid or neuromuscular blocker were recorded. One of the hemidiaphragm preparations was exposed to betamethasone 1 μmol litre^{-1} for the duration of the investigation. All changes of perfusate solution commenced with a 40-ml flush (the volume of the organ bath) followed by perfusate 2 ml min^{-1}. Control and betamethasone treated preparations were exposed to vecuronium at concentrations of 4, 6, 8 and 10 μmol litre^{-1}. Muscle contraction force (both direct and indirect) was recorded 30 min after each change of perfusate containing vecuronium. Vecuronium-free solutions were used to wash the preparations until twitch height returned to control values, at which time the next vecuronium concentration was added.

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 at the end of the experiment.

Statistics

Data from control and betamethasone groups were compared using analysis of covariance, with the dose of vecuronium as the covariate and terms for the control or betamethasone groups and for individual preparations within these groups. A test for difference in slope between the control group and betamethasone group was made by including a dose × group term in the model.
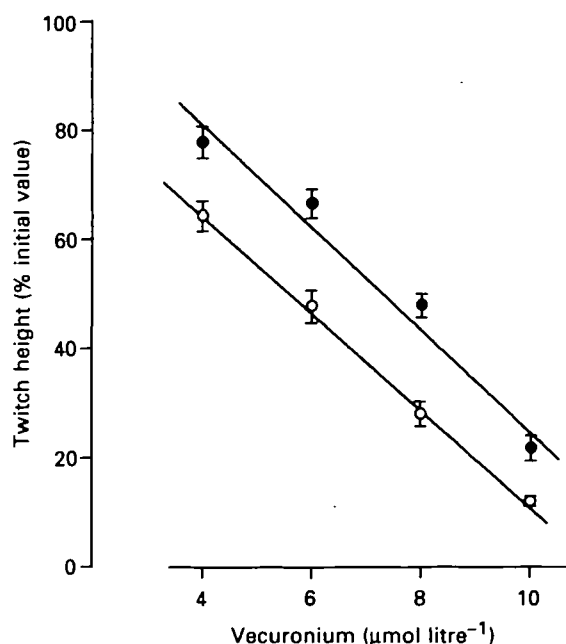

FIG. 1. Dose–response relationship for control (O) and betamethasone–pretreated (●) preparations (mean, SEM). The ED_{50} for 50 % depression of twitch height was 5.65 μmol litre^{-1} for control and 7.39 μmol litre^{-1} for betamethasone-pretreated preparations.

RESULTS

Satisfactory data were obtained from 20 hemidiaphragm preparations. Ten received betamethasone and 10 acted as control. No significant difference was observed in indirectly or directly stimulated mean twitch height before and after addition of betamethasone.

The slopes of the dose–response lines to vecuronium in control and betamethasone treated preparations were not significantly different ($P = 0.14$) (fig. 1). When a common slope was fitted, in

comparison with control, the betamethasone group showed significantly ($P = 0.0008$) less depression at all concentrations of vecuronium examined. The calculated ED_{50} for 50% depression of twitch height was 5.65 µmol litre^{-1} for controls and 7.39 µmol litre^{-1} for betamethasone pretreated preparations. Thus the addition of betamethasone increased the ED_{50} for vecuronium by approximately 30%.

DISCUSSION

In 1944, Torda and Wolff demonstrated that neuronal synthesis of acetylcholine could be increased by prior administration of adrenocorticotrophic hormone (ACTH) [6]. They demonstrated also that, in the absence of ACTH (hypophysectomized rats), there was reduced acetylcholine synthesis resulting in neuromuscular dysfunction, which was restored following administration of ACTH [7].

Recent case reports suggest that an interaction between corticosteroids and the neuromuscular junction may have clinical implications for the anaesthetist. There have been two case reports of partial recovery from pancuronium given in association with steroid therapy [1, 2] and a case of resistance to vecuronium in a patient receiving long-term testosterone [3]. Following two cases of unexpected movement during neurosurgery in patients receiving vecuronium and pretreated with betamethasone, we performed a retrospective review of 50 neurosurgical patients, examining the doses of vecuronium administered to patients with and without betamethasone pretreatment. Those data suggested that patients pretreated with betamethasone were resistant to the neuromuscular blocking properties of vecuronium and required, on average, 75% more blocking drug [4].

Animal studies that have examined the effects of tubocurarine in the presence of glucocorticoids have demonstrated resistance to the effects of the neuromuscular blocker in animals treated with prednisolone [8], dexamethasone [9], betamethasone [10] and triamcinolone [11, 12].

In this study we have demonstrated that the reduction in twitch tension caused by vecuronium was reduced considerably in the presence of betamethasone 1 µmol litre^{-1}. Although the mechanism of this interaction is not fully understood, previous work suggests several possible

sites where steroids may affect neuromuscular transmission:

(1) Motor neurone

Glucocorticoids have been shown to have a direct facilitatory effect at the impulse generating end of the motor nerve axon. In the cat, administration of large doses of methylprednisolone (8 mg kg^{-1} day^{-1} i.m.) for 1 week altered the electrical properties of spinal motor neurones and increased the excitability of the initial axon segment, the site of physiological impulse initiation [13].

(2) The presynaptic nerve terminal

Experimental work suggests that corticosteroids act presynaptically stimulating the synthesis, spontaneous release and stimulated release of acetylcholine.

(a) *Synthesis of acetylcholine.* The synthesis of acetylcholine is related directly to the supply of extraneural choline, which is transported by a sodium dependent carrier system located at the presynaptic membrane of the nerve terminal. Hemicholinium-3 is a presynaptic inhibitor of this carrier-mediated transport and virtually abolishes the synthesis of acetylcholine. Dexamethasone and prednisolone partially antagonize hemicholinium-3 [14] and increase acetylcholine synthesis *in vitro* by facilitating the uptake of choline and its subsequent incorporation into acetylcholine [15]. Electron micrographs of the neuromuscular junction support corticosteroid enhanced acetylcholine synthesis by revealing increases in the mean size of synaptic vesicles after incubation with prednisolone and dexamethasone [16].

(b) *Spontaneous release of acetylcholine.* Micromolar concentrations of prednisolone and dexamethasone have been shown to increase both the amplitude and frequency [17–19] of miniature endplate potentials. These observations suggest that corticosteroids facilitate spontaneous release of acetylcholine.

(c) *Stimulated release of acetylcholine.* The application of small concentrations of prednisolone (1 µmol litre^{-1}) to the frog neuromuscular junction results in a doubling of nerve evoked endplate currents. However, if prednisolone is added specifically to the end-plate via a micropipette,

such a response is not observed. This implies that stimulated release of acetylcholine may be augmented by corticosteroids acting at a presynaptic site [20].

(3) Postsynaptic nerve terminal

In the rat, large concentrations of prednisolone (0.6 mmol litre^{-1}) have a depressant effect on neuromuscular transmission. However, dexamethasone counteracts the suppression of contraction caused by tubocurarine at small concentrations, but this effect is not observed when greater concentrations of steroid are used [9]. It has been suggested, therefore, that the presynaptic facilitatory effects of small concentrations of corticosteroids on acetylcholine release may be overcome by a greater postsynaptic depressant effect at large concentrations [21]. This may explain why some workers have failed to demonstrate any evidence of recovery from tubocurarine after administration of prednisolone [22].

The evidence suggests that the major site of the facilitatory actions of corticosteroids at the neuromuscular junction is prejunctional and it seems feasible that the attenuation of competitive neuromuscular block observed in our study is a consequence of steroid-induced enhancement of acetylcholine synthesis and release.

A prejunctional reduction in release of acetylcholine by vecuronium, in addition to postjunctional receptor block, has been reported using in vitro toad neuromuscular preparations [23]. However, conclusions made from in vitro neuromuscular preparations investigating presynaptic modulation are limited [24] and the presence of autoregulating presynaptic acetylcholine receptors at the motor end-plate area has been questioned [25]. Perhaps of more clinical relevance are the observations of Baker and his colleagues in anaesthetized cats. They reported a biphasic prejunctional effect of vecuronium; at small concentrations of vecuronium, acetylcholine release was increased, while at greater concentrations acetylcholine release was reduced [26]. This may indicate that vecuronium interacts with two distinct populations of presynaptic acetylcholine receptors [24] or that the in situ effects of cholinergic antagonists (or agonists) on neuromuscular transmission are mediated, in part, by the central nervous system [25]. Whether the interaction between vecuronium and betamethasone occurs at presynaptic cholinergic receptors has not been demonstrated in this

report, but it would be of interest to examine further the interaction at the neuromuscular junction between corticosteroids and other cholinergic agonists–antagonists in terms of pre- and postjunctional effects.

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