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In-vitro Interaction between H_2 Antagonists and Vecuronium

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Abstract—The interaction between histamine H_2 antagonists and the neuromuscular blocking drug vecuronium was investigated in the rat phrenic nerve-hemidiaphragm preparation. Cimetidine alone, in the concentration range 800–4000 μM produced between 14 and 74% neuromuscular paralysis with an EC50 (mean ± s.e.) of 2900 ± 100 μM. Ranitidine augmented the indirectly-evoked muscle response at concentrations between 30 and 160 μM but at higher concentrations, between 300 and 1800 μM, produced neuromuscular paralysis. Famotidine produced negligible and statistically insignificant (0–5%) neuromuscular paralysis at concentrations between 0·3 and 300 μM. Cimetidine (800 μM) shifted the neuromuscular concentration-effect curve of vecuronium to the left in a parallel manner, while ranitidine (160 μM) shifted it to the right. The potentiation ratio was 1·90 ± 0·14 for cimetidine and 0·62 ± 0·05 for ranitidine. Famotidine (30 μM) did not alter the response to vecuronium. These data indicate that higher than clinically relevant concentrations of cimetidine and ranitidine produce neuromuscular paralysis and may potentiate the action of vecuronium. Low concentrations of ranitidine may antagonize the action of vecuronium. Famotidine, in contrast, lacks significant neuromuscular effects.

H_2 Antagonists are used as anaesthetic pre-medication to minimize the risk of aspiration pneumonitis in high-risk surgical patients (Alpert et al 1989). Cimetidine, the prototypic agent, has been commonly used for this purpose but ranitidine and more recently, famotidine, the more potent and selective agent, are becoming more popular.

In patients who receive H_2 antagonists as pre-medication, there is potential for interaction with the neuromuscular blocking drugs which are administered routinely for tracheal intubation and muscle relaxation during surgery. This is due to the well-documented effects of cimetidine and ranitidine on neuromuscular transmission (Gwee & Cheah 1986) and lack of such effects from famotidine (Kosh et al 1989). Several studies have examined this potential interaction in patients using different H_2 antagonists. Oral cimetidine or ranitidine does not influence the duration of vecuronium-induced neuromuscular paralysis in post-partum patients (Hawkins et al 1989), but intra-muscular cimetidine increases the duration of vecuronium-induced neuromuscular paralysis in patients undergoing short gynaecological surgery (Ormezzano et al 1988). An interaction between cimetidine and vecuronium involving potentiation of blockade but not between ranitidine and vecuronium has recently been confirmed clinically (McCarthy et al 1991). In contrast, cimetidine potentiates (Mishra & Ramzan 1992) and ranitidine antagonizes (Law et al 1989) the action of another neuromuscular blocker, atracurium, in-vivo in rats. An interaction between atracurium and cimetidine is not, however, seen in patients (McCarthy et al 1991) presumably due to the low concentrations of cimetidine achieved in patients compared with that in rats. The neuromuscular effects of famotidine have not been examined clinically.

The present experiments were undertaken to investigate the neuromuscular actions of three H_2 antagonists currently used in anaesthesia.

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Materials and Methods

The experiments were carried out on rat phrenic nerve-hemidiaphragm preparations (Bulbring 1946) which were prepared from 250 to 450 g male Sprague-Dawley rats, and mounted in 50-mL organ baths containing Krebs solution (mM): Na^+ 138, K^+ 5·9, Ca^{2+} 2·5, Mg^{2+} 1·22, Cl^- 123, $H_2PO_4^-$ 1·2, HCO_3^- 25, glucose 115, maintained at 37°C and bubbled with 5% CO_2 in O_2. The nerve was stimulated at intervals of 0·5 s for 2 s (2 Hz) with rectangular supramaximal pulses of 0·2 ms duration repeated every 10 s (train-of-four stimulation pattern). The resulting isometric muscle contractions were recorded using a force-displacement transducer (Grass Instruments, Model FT03) and a chart recorder (Watanabe Instruments, Miniwriter). The first response of the train (T1) was used to describe the neuromuscular block or paralysis.

All experiments were commenced after 30 min equilibration. Drug solutions were freshly prepared by dilution of commercial injections (vecuronium or H_2 antagonists) or by dissolution of the pure drug powder (H_2 or H_3 antagonists or H_3 antagonist) in Krebs buffer. The injection vehicles for vecuronium or the H_2 antagonists do not affect muscle twitch response (Law et al 1989; Mishra & Ramzan 1992). These drugs were added to the organ bath in volumes of 100–500 μL and dispersed rapidly. The twitch response was allowed to stabilize for 20 min after addition of each drug concentration, i.e. a concentration cycle of 20 min was used.

In the first experiment, four preparations were used to construct the neuromuscular concentration-response curve for vecuronium. Three additions of vecuronium to achieve final vecuronium bath concentrations of between 3 and 12 μM respectively to elicit approximately 25, 50 and 75% neuromuscular paralysis were used.

A neuromuscular concentration-effect curve for each H_2 antagonist was also constructed in a similar manner using cimetidine 800, 1600, 2400 and 4000 μM; ranitidine 160, 300,

600 and 1800 μM or famotidine 0·3, 3, 30 and 300 μM. These concentrations were chosen to reflect those eliciting interactions in-vivo in rats (Law et al 1989; Mishra & Ramzan 1992). Four hemidiaphragm preparations were used for each H_2 antagonist. A subliminal concentration of each H_2 antagonist, i.e. the minimum concentration that produced neuromuscular paralysis was determined from this data. In these preparations, the effect of 10 mM Ca^{2+} on the H_2-antagonist-induced neuromuscular paralysis was also determined at the end of the experiment.

In the third set of experiments, the neuromuscular paralysis was assessed first in the presence of vecuronium only between 3 and 12 μM followed 20 min later with either cimetidine (800 μM), ranitidine (160 μM) or famotidine (30 μM). Thus the effect of these concentrations of the H_2 antagonists was assessed against the effect produced by EC25, EC50, or EC75 concentrations of vecuronium. Four preparations were used for each concentration of vecuronium with each H_2 antagonist.

In the final series of experiments, the possible mechanism of these interactions was examined by addition of either an H_2 (dimaprit, 214 μM) or H_3 ((*R*)-α-methyl histamine, 25 μM) agonist or an H_3 antagonist (thioperamide, 12 μM) to determine if the effects of cimetidine, ranitidine or famotidine were mediated via H_2 or H_3 receptors. These concentrations reflect their effective concentrations at these histaminergic receptor sites (Hill 1992).

Concentration-response relationships were constructed for vecuronium or each H_2 antagonist alone or for vecuronium in the presence of each H_2 antagonist. For the neuromuscular blocking activity of cimetidine and ranitidine respectively, these relationships were fitted using nonlinear regression techniques to a sigmoid Hill equation to obtain estimates of the concentration (EC50) eliciting 50% neuromuscular paralysis (Mishra & Ramzan 1992). The vecuronium concentration response data were fitted to log-linear equations using linear regression techniques. The significance of such relationships was tested by analysis of variance. The slopes of these relationships with or without each H_2 antagonist were available directly from the fits. The bath concentration corresponding to 50% neuromuscular paralysis (EC50) and its 95% confidence intervals were then calculated. The ratio of neuromuscular paralysis before and after the addition of H_2 antagonist was expressed as the potentiation ratio, which was measured as the displacement of the vecuronium concentration-response curve along the X-axis, i.e. the ratio of the EC50 values for vecuronium in the presence and absence of H_2 antagonist. The neuromuscular paralysis before and after addition of H_2 antagonist were also compared by paired Student's *t*-test. Statistical significance was defined at $P<0·05$. All data are expressed as mean ± s.e. with 95% confidence intervals where appropriate.

Results

Concentrations of cimetidine below 800 μM failed to alter neuromuscular transmission. At 1600 μM, a partial (17 ± 4%) neuromuscular paralysis was produced and at 4000 μM the paralysis was 74 ± 3%. In contrast, ranitidine produced a 27 ± 6% augmentation of the muscle twitch response at 160 μM but elicited neuromuscular paralysis at concentrations above 300 μM. At the highest ranitidine concentration of 1800 μM, the neuromuscular paralysis was 47 ± 6%. Addition of 10 mM calcium to the preparation completely reversed the neuromuscular paralysis elicited with either cimetidine or ranitidine. Famotidine failed to show any effect on neuromuscular transmission at bath concentrations of 0·3, 3 and 30 μM but at 300 μM, a small (6 ± 5%) but statistically insignificant ($P>0·05$) neuromuscular paralysis was observed. The neuromuscular concentration-effect relationships for each H_2 antagonist alone are summarized in Fig. 1.

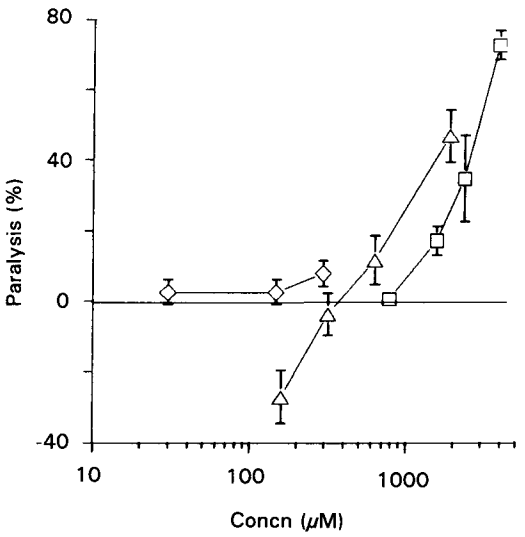

FIG. 1. Relationship between H_2 antagonist bath concentration and neuromuscular paralysis induced by cimetidine (□), ranitidine (△) or famotidine (◇). Negative neuromuscular paralysis represents augmentation (rather than suppression) of normal twitch response.

Vecuronium produced neuromuscular paralysis in the phrenic nerve-hemidiaphragm preparation in the concentration range 4–12 μM. The EC50 for vecuronium in this preparation was 6 μM. Cimetidine, 800 μM, potentiated vecuronium-induced paralysis which stabilized after approximately 15 min, while ranitidine at 160 μM antagonized or reversed the paralysis within 2 min. Famotidine, in contrast, did not show any effect on vecuronium-induced paralysis. These varied effects of cimetidine, ranitidine and famotidine on vecuronium-induced paralysis are illustrated by the muscle twitch recordings in Fig. 2.

The slopes (and 95% confidence intervals) of the vecuronium response-concentration relationships with or without cimetidine were 106% (65–146) vs 82% (58–105). The slopes in the absence and presence of ranitidine were 229% (207–251) vs 223% (151–293). Thus cimetidine, 800 μM, produced a parallel shift to the left while ranitidine, 160 μM, a parallel shift to the right in the concentration-response relationship for vecuronium. The EC50 for vecuronium in the presence of cimetidine was 3 μM compared with 6·4 μM without cimetidine ($P<0·05$). Ranitidine increased vecuronium's EC50 value from 7·1 to 8·9 μM ($P<0·05$). The potentiation factor for cimetidine was 1·9 ± 0·14 while that for ranitidine was less than unity (0·62 ± 0·05), indicating reversal rather than potentiation. Famotidine did not alter either the slope (pre- vs post-famotidine, 88 vs 82%) of the vecuronium concentration-effect relationship or its EC50 (pre- and post-famotidine 6·4 vs 6 μM, $P>0·05$). The neuromuscular concentration-

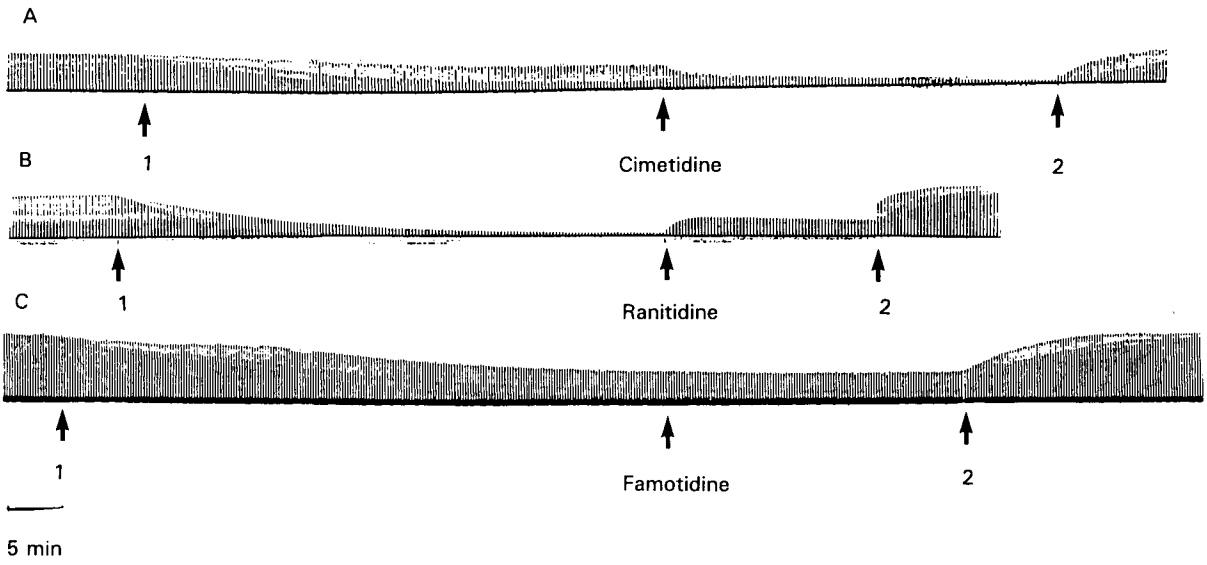

FIG. 2. Effect of cimetidine, ranitidine or famotidine on vecuronium-induced neuromuscular paralysis in the rat phrenic nerve-hemidiaphragm preparation. Cimetidine potentiates, ranitidine antagonizes and famotidine has no effect on vecuronium paralysis.

response relationships for vecuronium in the presence and absence of either cimetidine, ranitidine or famotidine are presented in Fig. 3. The corresponding EC50 values for vecuronium with and without the H_2 antagonists are summarized in Table 1.

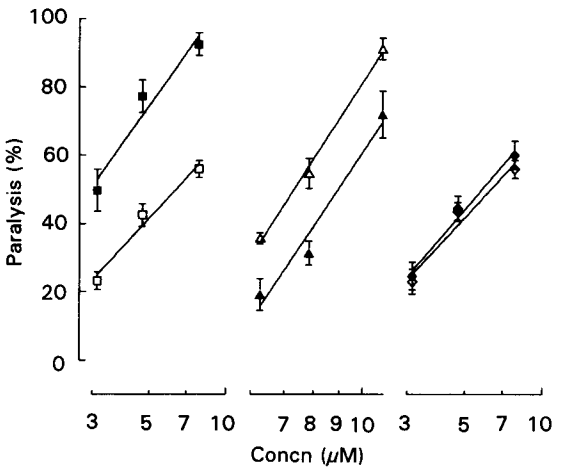

FIG. 3. Relationship between neuromuscular paralysis and vecuronium bath concentration before (open symbols) and after (solid symbols) addition of cimetidine 800 μM (■, □), ranitidine 160 μM (▲, △) or famotidine 30 μM (◆, ◇).

Table 1. Effect of H_2 antagonists on vecuronium bath concentrations causing 50% neuromuscular paralysis (EC50) in-vitro in rats.

	Vecuronium EC50 (μM)		
	Cimetidine (800 μM)	Ranitidine (160 μM)	Famotidine (30 μM)
Control	6·4 (4·5–8·2)	7·1 (6·4–7·8)	6·4 (4·2–8·7)
Treatment	3·0* (1·8–4·1)	8·9* (6·0–11·7)	6·0 (4·1–7·9)

Each value represents estimate with 95% C.I. in parentheses.
* $P < 0.05$ compared with corresponding control.

Pre-treatment of the nerve-muscle preparations with appropriate concentrations of either specific agonists for the H_2 or H_3 receptor or a specific H_3 antagonist did not affect the baseline neuromuscular transmission or the neuromuscular blocking activity of either cimetidine or vecuronium.

Discussion

The neuromuscular paralysis produced by high concentrations of cimetidine (and ranitidine) are consistent with previous in-vitro findings (Galatulas et al 1980; Bossa et al 1982) and may reflect intrinsic neuromuscular-blocking action resulting from competition with prejunctional calcium (Galatulas et al 1980). The surprising finding was that ranitidine augmented the twitch response at low concentrations but blocked neuromuscular transmission at high concentrations. The neuromuscular paralysis produced by ranitidine (and cimetidine) at higher concentrations was reversed by calcium chloride suggesting Ca^{2+} antagonism at presynaptic receptor sites. Whether ranitidine increases Ca^{2+} influx at low concentrations is not clear from this study, but the augmentation of twitch response by ranitidine is consistent with its anti-cholinesterase activity. There is also the possibility that augmentation of control twitch response is a net result of increase in Ca^{2+} influx and anti-cholinesterase activity at low concentrations of ranitidine (Hansen & Bertl 1983a). Lack of a similar augmentation by cimetidine may reflect its low anti-cholinesterase potency compared to ranitidine (Hansen & Bertl 1983b). Famotidine had a negligible effect on neuromuscular transmission and this is consistent with its weak acetylcholinesterase inhibition (Aono et al 1986; Kosh et al 1989) and lack of significant direct effects at the neuromuscular junction (Re & Di Sarra 1989).

The parallel shift to the left in the vecuronium concentration-response curve with cimetidine and to the right with ranitidine provide further evidence that cimetidine produces neuromuscular blockade and ranitidine inhibits acetylcholinesterase at subliminal concentrations. Lower neuromuscu-

lar-blocking concentrations of vecuronium will be needed in the presence of cimetidine, while ranitidine will increase acetylcholine concentrations leading to more effective competition with vecuronium and reversal of paralysis.

Use of specific ligands for both H_2 and H_3 histamine receptors failed to affect neuromuscular transmission or alter the pattern of neuromuscular paralysis induced by cimetidine or ranitidine. The neuromuscular action of the H_2 antagonists is unlikely, therefore, to be mediated via the H_2 or H_3 receptors and presumably reflects their varying direct effects at the neuromuscular junction or the acetylcholinesterases in the synaptic cleft. Calcium is important in the neuromuscular blocking activity of cimetidine and ranitidine and can reverse their effects. This is consistent with previous findings (Galatulas et al 1980).

This in-vitro study confirms the in-vivo data in rats demonstrating that cimetidine potentiates, and ranitidine antagonizes, the activity of non-depolarizing neuromuscular blockers (Law et al 1989; Mishra & Ramzan 1992). However, these effects do not occur at clinically relevant concentrations. Cimetidine produces neuromuscular effects by itself at concentrations which are approximately 20-fold those (16–32 μM) noted in patients (Walkenstein et al 1978) and potentiation of vecuronium's response is not evident until cimetidine concentrations are ten times clinical concentrations. At first sight, the finding that concentrations of cimetidine (and ranitidine) are higher than those noted clinically may make the interactions observed in the present study appear to be clinically irrelevant. However, the concentrations of the neuromuscular blocker required to produce paralysis in-vitro are also very much higher than those seen in patients. For example, in the present study, 90% paralysis was elicited with 12 μM vecuronium yet in patients 90% paralysis is produced with approximately 0·8 μM. Thus, the in-vitro replication of a clinical interaction between cimetidine and vecuronium requires relatively high concentrations of both drugs. This may explain why, in patients, either a potentiation (McCarthy et al 1991) or no effect (Hawkins et al 1989) has been reported when cimetidine pre-medication doses have been used in relatively fit patients. It is likely, however, that where neuromuscular transmission reserve is reduced, such interactions may reach clinical significance. Ranitidine neuromuscular-blocking activity or reversal of vecuronium paralysis is also unlikely to be seen in patients exhibiting the therapeutic range (1·3–13 μM) of ranitidine concentrations (Garg et al 1983). At this stage, no clinical interaction has been observed between ranitidine and vecuronium (McCarthy et al 1991), but one should not be ruled out, particularly if ranitidine is administered during anaesthesia or in patients with impaired renal function who would be expected to have reduced clearance of ranitidine. Famotidine would have the lowest interaction potential with neuromuscular blockers at clinically relevant concentrations of 0·015–0·15 μM (Ohinisi 1990).

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