Laboratory Investigations

Interaction between succinylcholine and ranitidine in rats

Yogendra Mishra M Pharm, Iqbal Ramzan PhD

The hypothesis that the histamine H₂ receptor blocker ranitidine potentiates neuromuscular paralysis during anaesthesia was tested in vivo in urethane anaesthetised and mechanically ventilated rats. Succinylcholine was administered as a bolus and constant-rate infusion to maintain 48.5% (± 2.5 SEM) tibialis anterior muscle paralysis in 14 rats. Ranitidine 2.5, 5, 10, or 20 mg $\cdot$ kg⁻¹ iv, was then administered into groups of three or five rats. Ranitidine produced an immediate potentiation of neuromuscular paralysis followed by a transient reversal and then a continued steady-state potentiation. Peak potentiation occurred within 20 (± 3.3) sec and was maintained in all the rats to steady-state. Peak reversal was evident 70 (± 8.1) sec after ranitidine administration. There was an excellent relationship ($r^2 = 0.98$, $P < 0.001$) between peak potentiation and serum ranitidine concentration with 50% potentiation occurring at 25.8 (± 1.1) μ g $\cdot$ ml⁻¹. There was a weak relationship ($r^2 = 0.39$, $P < 0.05$) between peak reversal and serum ranitidine but potentiation at steady-state was not correlated to serum ranitidine concentration ($r^2 = 0.19$, $P > 0.05$). These results show that ranitidine alters the neuromuscular action of succinylcholine in rats in a similar manner to cimetidine.

Cette étude vise à vérifier l'hypothèse selon laquelle l'antagoniste des récepteurs H₂, la ranitidine potentialise la paralysie neu-

romusculaire pendant l'anesthésie. Cette étude est réalisée in vivo sur des rats anesthésiés à l'uréthane et ventilés mécaniquement. La succinylcholine est administrée en dose simple suivie d'une perfusion continue de façon à maintenir à 48% ($\pm 2,5$ SEM) la paralysie du muscle tibial antérieur du rat. La ranitidine 2, 5, 10, ou 20 mg $\cdot$ kg⁻¹ est administrée à des groupes de trois ou cinq rats. La ranitidine produit immédiatement une potentialisation de la paralysie neuromusculaire suivie d'un renversement temporaire et, par la suite, d'une potentialisation continue une fois l'état d'équilibre atteint. Une potentialisation de pointe survient en-deça de 20 ($\pm 3,3$) sec est maintenue chez tous les rats jusqu'à l'atteinte de l'état d'équilibre. Un renversement maximal est évident à 70 ($\pm 8,1$) sec après l'administration de la ranitidine. On retrouve une excellente relation ($r^2 = 0,98$, $P < 0,001$) entre la potentialisation maximum et le taux de ranitidine sérique lorsque la potentialisation à 50% survient à 25,8 ($\pm 1,1$) μ g $\cdot$ ml⁻¹. La relation entre le renversement maximum et le taux sérique de ranitidine ($r^2 = 0,39$, $P > 0,05$) est faible. Par contre, il n'y a pas de corrélation de l'état d'équilibre avec le taux sérique de ranitidine ($r^2 = 0,19$, $P < 0,05$). Ces résultats démontrent que la ranitidine altère l'activité neuromusculaire de la succinylcholine chez les rats de la même façon que la cimétidine.

Key words

NEUROMUSCULAR RELAXANTS: succinylcholine;
HISTAMINE: ranitidine;
PHARMACOLOGY: interaction.

From the Pharmacy Department, The University of Sydney, New South Wales, Australia.

Address correspondence to: Dr. I. Ramzan, Pharmacy Department, The University of Sydney, New South Wales 2006, Australia.

This project was supported in part by financial assistance from the Pharmacy Research Trust of New South Wales.

Accepted for publication 27th September, 1992.

Histamine H₂ receptor antagonists are used to increase pH and decrease the volume of gastric contents before induction of anaesthesia to minimize aspiration pneumonitis.¹ Cimetidine, the prototypic H₂ antagonist, has been commonly used for this purpose in the past but recently ranitidine has become popular. Normally, ranitidine, 150 mg, is administered *po* the night before surgery and one to two hours before induction of anaesthesia. Since ranitidine exhibits diverse cholinergic effects including an anti-cholinesterase and a neuromuscular blocking effect,² there is potential for interaction between ranitidine and the neuromuscular blocking drugs administered to facilitate intubation and to maintain muscle relaxation during surgery.

The effect of ranitidine on the action of the neuromuscular blocking drugs has been examined previously in patients receiving oral premedication doses of the drug. The duration of action of the non-depolarizing blockers, vecuronium and atracurium was not affected by ranitidine.³ Similarly, ranitidine failed to alter the action of the depolarizing agent succinylcholine in patients.⁴ In contrast, in rats, ranitidine *iv* reversed an intense atracurium-induced neuromuscular paralysis⁵ while *intravenous* cimetidine *iv* had a triphasic action against succinylcholine-induced neuromuscular paralysis which included potentiation.⁶

The present study was designed to examine the potential interaction between ranitidine and succinylcholine in rats and to test the hypothesis that ranitidine affects neuromuscular function during anaesthesia in a similar manner to cimetidine.

Methods

Anaesthesia and surgical preparation

The protocol was approved by the Animal Care and Use Committee of The University of Sydney. Eighteen male Sprague-Dawley rats (University of Sydney Animal Services) weighing 328–453 g were used. Anaesthesia was induced with diethyl ether and maintained throughout the experiment with *iv* urethane, 1.25 g · kg⁻¹ in total, given as six divided doses during the surgical preparation of the animal. Rectal temperature was servo-controlled to approximately 38° C with a heated surgical table and a heat lamp.

The surgical preparation consisted of cannulation of both external jugular veins and a carotid artery. The right jugular vein was for urethane and ranitidine administration while the left jugular vein was for the administration of succinylcholine. Blood samples for ranitidine assay were obtained from the carotid artery cannula. Following the vascular cannulations and tracheostomy, the lungs of each animal were ventilated mechanically with room air. A sciatic nerve in the thigh was then isolated and stimulated electrically using supramaximal stimuli of 2 volts with a duration of 0.2 msec at a frequency of 0.1 Hz. The mechanical twitch response of the isolated tibialis anterior muscle was then monitored using a force-displacement transducer and polygraph recorder.

Experimental protocol

The experimental design was similar to that used previously in this laboratory to examine the interaction between cimetidine and succinylcholine in rats.⁶ The preparation was allowed to stabilize for 15 min during which baseline twitch measurements were made. Succinylcholine was then administered as an *iv* bolus of 1 mg · kg⁻¹,

given as a 1 mg · ml⁻¹ saline solution equivalent to 1 ml · kg⁻¹ of injection solution. An infusion of succinylcholine was then started at approximately 50 µg · kg⁻¹ · min⁻¹ and titrated to maintain approximately 50% neuromuscular paralysis. The drug concentration in the infusate was chosen to deliver about 1.5 ml of solution every hour. After a further 20 min of stabilization, ranitidine *iv* was administered into groups of three or five rats in a random order in doses of 2.5, 5, 10 or 20 mg · kg⁻¹ using a diluted commercial ranitidine hydrochloride injection (Zantac[®]) in normal saline (1 ml · kg⁻¹ of solution). The selected doses were considered to be sufficient to show either zero or a maximum effect and were chosen based on a previous interaction study of ranitidine and atracurium in rats.⁵ Two blood samples, each 0.35 ml, were withdrawn, one as close as possible to the time of the peak potentiation and peak reversal by ranitidine and the other when the potentiation by ranitidine had stabilized. After observation of the entire cholinergic effect of ranitidine, approximately 20–25 min after its dosing, the succinylcholine infusion was discontinued to record the spontaneous recovery of the muscle twitch response. In another four rats, either 2.5 or 20 mg · kg⁻¹ ranitidine were given *iv* but without any previous succinylcholine to see if ranitidine by itself could alter neuromuscular transmission.

Measurements and calculations

Ranitidine serum concentrations were determined using a modification of a published reversed phase liquid chromatographic procedure.⁷ Briefly, this involved addition of 10 µl of trimethoprim (10 mg · ml⁻¹) as internal standard and extraction of 50 µl samples with 4% isopropanol in ethylacetate (0.5 ml) and liquid chromatography of the dry extracted residue. The mobile phase consisted of 10% acetonitrile in 5 mM phosphate buffer (pH 3). The coefficient of variation of the assay was 1.1 and 0.5% at 0.5 and 10 µg · ml⁻¹ respectively. The minimum detectable concentration was 0.1 µg · ml⁻¹ using a 100 µl volume of serum.

Ranitidine potentiation and reversal of succinylcholine-induced neuromuscular paralysis was calculated as:

$$\text{Potentiation of paralysis} = \frac{\text{Pre-ranitidine twitch height} - \text{Post-ranitidine twitch height (at peak or plateau potentiation)}}{\text{Pre-ranitidine twitch height}} \times 100$$

and

$$\text{Reversal of paralysis} = \frac{\text{Pre-ranitidine paralysis} - \text{Post-ranitidine paralysis (at reversal)}}{\text{Pre-ranitidine paralysis}} \times 100$$

FIGURE 1 Muscle twitch recording in a rat illustrating the effects of *iv* ranitidine. 1 = steady-state neuromuscular paralysis, 2 = peak potentiation, 3 = transient reversal, 4 = steady-state potentiation. At the first arrow ranitidine, $20 \text{ mg} \cdot \text{kg}^{-1}$, was administered and the succinylcholine infusion was discontinued at the second arrow.

These effects (either peak or plateau potentiation or peak reversal) were related to either the ranitidine dose administered or the ranitidine serum concentration measured using either a linear, log-linear, an E_{max} or a sigmoid E_{max} (Hill) equation using the personal computer based non-linear regression program PCNONLIN (Version 3.0, SCI Software, U.S.A.).⁶ The equation that best characterized the relationship between the effect of ranitidine and its concentration was chosen and the statistical significance determined, as described previously for the interaction between cimetidine and succinylcholine.⁶ All data are presented as mean $\pm$ SEM.

Results

Succinylcholine mean ($\pm$ SEM) infusion rate of $35.4 \pm 2.4 \text{ } \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ produced $48.5 \pm 2.5\%$ neuromuscular paralysis in 14 rats. When *iv* ranitidine was administered it produced an immediate potentiation of paralysis in all rats. This was followed by a transient reversal of paralysis in all animals. A second phase of potentiation was then noted in every rat which plateaued or attained a steady-state as judged by $<5\%$ change in twitch height during a five-minute interval. This steady-state potentiation ranged from 11 to 89%. The onset of peak potentiation occurred in $20.0 \pm 3.3 \text{ sec}$ after ranitidine dosing while the peak reversal was observed $70.0 \pm 8.1 \text{ sec}$ after the dose. The second phase of potentiation took $23.3 \pm 1.4 \text{ min}$ to stabilize. The observed triphasic effects of *iv* ranitidine are depicted in the muscle twitch tracing presented in Figure 1.

The first blood sample for ranitidine assay was taken $46 \pm 3 \text{ sec}$ after the ranitidine dose and the ranitidine concentrations varied between 8 and $67 \text{ } \mu\text{g} \cdot \text{ml}^{-1}$ for ranitidine doses between 2.5 and $20 \text{ mg} \cdot \text{kg}^{-1}$. This sampling time was delayed by 26 sec compared with the time of peak potentiation due to technical constraints. Similarly, no ranitidine concentration was available exactly at the time of peak reversal with the first blood sample being taken 24 sec before peak reversal was observed. The second ranitidine concentrations were determined when the potentiation by ranitidine had stabilised; these concentrations ranged from 0.7 to $12 \text{ } \mu\text{g} \cdot \text{ml}^{-1}$.

There was an excellent relationship between the degree of peak potentiation of neuromuscular paralysis by ranitidine and its dose ($r^2 = 0.98$, $P < 0.001$) and serum concentration ($r^2 = 0.98$, $P < 0.001$) respectively and the sigmoid E_{max} (or Hill) equation was the most appropriate for characterizing these relationships. The ranitidine dose that produced 50% potentiation during its peak effect was $9.2 \pm 0.4 \text{ mg} \cdot \text{kg}^{-1}$ and was associated with a ranitidine serum concentration $25.8 \pm 1.1 \text{ } \mu\text{g} \cdot \text{ml}^{-1}$ (or $82.1 \pm 3.5 \text{ } \mu\text{M}$). Maximal (peak) reversal, or pre-ranitidine paralysis, was between 13 and 56%. There was a significant but weak relationship between the peak reversal of paralysis and the dose ($r^2 = 0.49$, $P < 0.05$) and serum ranitidine concentration ($r^2 = 0.39$, $P < 0.05$) respectively, with linear equations being most appropriate. Potentiation as steady-state was not related to either the ranitidine dose ($r^2 = 0.13$, $P > 0.05$) or its serum concentration ($r^2 = 0.19$, $P > 0.05$) but neuromuscular paralysis at the time of steady-state potentiation after ranitidine dosing was significantly higher ($P < 0.001$, paired *t* test) than pre-ranitidine neuromuscular paralysis in each rat (Figure 2). The relationships between the three effects (peak potentiation, peak reversal and steady-state potentiation) of ranitidine and its dose and serum concentrations respectively are depicted in Figures 3, 4 and 5.

When ranitidine 2.5 or $20 \text{ mg} \cdot \text{kg}^{-1}$ was administered alone to rats who had not received any succinylcholine, there was no effect on the baseline twitch response.

Discussion

Ranitidine, like cimetidine, produced three distinct cholinergic effects against the neuromuscular paralysis induced with the depolarizing agent succinylcholine in rats. Peak potentiation of paralysis was followed by a rapid peak reversal which preceded plateau or steady-state potentiation. The predominant effect of ranitidine, however, was to potentiate the neuromuscular paralysis and the peak reversal was transient. This was confirmed by the observation of a strong relationship between peak potentiation and ranitidine serum concentration even though these concentrations were determined approximately 30 sec after peak potentiation had been observed. In con-

FIGURE 2 Succinylcholine-induced neuromuscular paralysis before and after *iv* ranitidine doses ranging from 2.5 to 20 $\text{mg} \cdot \text{kg}^{-1}$. Steady-state paralysis after ranitidine dosing was significantly higher ($P < 0.001$) than pre-ranitidine steady-state paralysis.

trast, reversal of paralysis was only weakly correlated to these same serum concentrations, although these concentrations did not correspond exactly to the time of peak reversal and were taken about 30 sec earlier.

The peak potentiation of neuromuscular paralysis by ranitidine may have resulted from an intrinsic neuromuscular blocking effect of ranitidine since ranitidine produces neuromuscular blockade of high doses (30–60 $\text{mg} \cdot \text{kg}^{-1}$).² However, in this present study, ranitidine alone at 20 $\text{mg} \cdot \text{kg}^{-1}$ failed to affect neuromuscular transmission. This was not surprising since serum concentrations of ranitidine generated with this dose were approximately 23 $\mu\text{g} \cdot \text{ml}^{-1}$ which were substantially lower than the ranitidine concentration (500–1000 $\mu\text{g} \cdot \text{ml}^{-1}$) needed to elicit neuromuscular blockade *in vitro*.² Peak potentiation observed here with ranitidine may, however, have resulted from its intrinsic neuromuscular blocking activity even at the low doses used since succinylcholine was also present at neuromuscular blocking concentrations and would have reduced the margin of safety of neuromuscular transmission. The anti-acetylcholinesterase (AChE) effect of ranitidine which would have been present at the doses and concentrations of ranitidine used here,^{2,9} would also contribute to the observed peak potentiation by elevating acetylcholine concentrations with a consequent potentiation of a depolarizing succinylcholine block. Peak potentiation therefore may have been a net result of an intrinsic neuro-

FIGURE 3 Relationship between ranitidine dose and serum concentration respectively and the peak potentiation of succinylcholine-induced neuromuscular paralysis in rats. These relationships were most appropriately characterized by use of a sigmoidal Emax (Hill) equation. The predicted relationships together with the mean and SEM (dose) or individual data (concentration) are presented.

muscular blocking effect as well as an anti-AChE effect of ranitidine. This may be the reason why ranitidine produced net reversal (and not net potentiation) against atracurium⁵ since the predominant anti-AChE effect of ranitidine would reverse rather than potentiate a non-depolarizing blockade induced with atracurium.

The transient peak reversal of neuromuscular paralysis produced by ranitidine may have resulted from the known anti-acetylcholinesterase (AChE) effect of the drug⁹ provided there was a phase II (non-depolarizing or competitive) succinylcholine block. Phase II block would be reversed by increased acetylcholine concentrations resulting from AChE inhibition by ranitidine. Alternatively, the depolarizing (or phase I) block may have converted to a non-depolarizing (or phase II) block in the presence of ranitidine. The presence of succinylcholine phase II block or a transition in nature of the block could not be confirmed in this study since the single twitch response used was unable to distinguish between a phase I and a phase II neuromuscular blockade. It is interesting, however, that using the same experimental set-up, edrophonium, a known anti-AChE agent was able to reverse (but following peak potentiation) succinylcholine-induced paralysis at a dose of 20 $\text{mg} \cdot \text{kg}^{-1}$ in several rats (data not presented here), suggesting presence of a mixed block.

The reason for the steady-state potentiation of succinylcholine-induced neuromuscular paralysis by ranitidine is more difficult to explain. It is not related to ranitidine dose or serum concentration, and by implication it may be argued that ranitidine is not causing it. One possibility is that the steady-state potentiation results from butyrylcholinesterase (BuChE) inhibition. Since succinylcholine depends on hydrolysis by BuChE for termination of its

FIGURE 4 Relationship between ranitidine dose and serum concentration respectively and the transient reversal of succinylcholine-induced neuromuscular paralysis in rats. These relationships were most appropriately characterized by use of linear equations. The predicted relationships together with the mean and SEM (dose) or individual data (concentration) are presented.

action, an inhibition of this enzyme would cause succinylcholine concentrations to accumulate during a constant infusion leading to a more intense blockade. Ranitidine is in fact a more potent inhibitor of AChE than BuChE but the concentrations required for inhibition of AChE and BuChE overlap.⁹ The pattern of potentiation leading to a plateau or steady-state supports slow accumulation of succinylcholine due to decreased BuChE activity so that succinylcholine infusion which was titrated to elicit ~50% paralysis before ranitidine administration produced significantly greater than 50% steady-state paralysis. One other possibility is that the potentiation at steady-state is due to muscle fatigue; especially in the presence of continued anti-cholinesterase effect of ranitidine. This cannot be ruled out entirely but is unlikely to be the only reason since the muscle preparations returned to final twitch heights of $87 \pm 3\%$ of the original (control) twitch height, illustrating minimal muscle fatigue. In any case, the degree of steady-state potentiation (11 to 89%) was too great to be explained entirely by muscle fatigue. Similarly, withdrawal of blood samples (leading to an insignificant loss of plasma BuChE) cannot account for the steady-state potentiation as no potentiation was seen in control experiments substituting ranitidine with normal saline.

The neuromuscular effects of ranitidine observed in this study against succinylcholine in rats are qualitatively similar to the effects of *iv* cimetidine against succinylcholine-induced neuromuscular paralysis in rats observed previously in this laboratory.⁶ Cimetidine and ranitidine both produce the same range of peak potentiation, transient peak reversal followed by steady-state potentiation of succinylcholine-induced paralysis. This implies that

FIGURE 5 Relationship between ranitidine dose and serum concentration respectively and the steady-state potentiation of succinylcholine-induced neuromuscular paralysis in rats. These relationships were not statistically significant. The mean and SEM (dose) or individual data (concentration) are presented.

both these agents interact with succinylcholine via the same mechanism(s) and this is consistent with their three distinct (direct neuromuscular block and AChE and BuChE inhibition) cholinergic actions.² These two H_2 -antagonists, however, differ in their potency in producing these effects; ranitidine has a two-fold lower EC_{50} for peak potentiation and reversal respectively than cimetidine.

The overall potentiation of succinylcholine's effect by ranitidine is not likely to be pronounced in quantitative terms and may be unnoticed in clinical anaesthesia since ranitidine doses used in patients are somewhat lower. In addition, the serum concentrations (8 to $67 \mu\text{g} \cdot \text{ml}^{-1}$) that produce potentiation in rats are substantially higher than that noted in patients after intravenous ($4 \mu\text{g} \cdot \text{ml}^{-1}$) or oral ($0.2 \mu\text{g} \cdot \text{ml}^{-1}$) dosing.¹⁰ Also, with clinical premedication dose, ranitidine does not affect BuChE enzyme activity *in vivo*.⁴ However, caution will need to be exercised in patients who are otherwise at risk of displaying prolonged paralysis, for example, those with abnormal or low cholinesterase enzyme activity or those receiving drugs that depress neuromuscular transmission.

In conclusion, this study demonstrates that *iv* ranitidine alters the action of succinylcholine in rats in a manner similar to that noted with *iv* cimetidine.

References

- 1 Coombs DW, Hooper D, Colton T. Pre-anesthetic cimetidine alteration of gastric fluid volume and pH. *Anesth Analg* 1979; 58: 183-8.
- 2 Gwee MCE, Cheah LS. Actions of cimetidine and ranitidine at some cholinergic sites: implications in toxicology and anesthesia. *Life Sci* 1986; 39: 383-8.
- 3 McCarthy G, Mirakhor RK, Elliott P, Wright J. Effect of

- H₂-receptor antagonist pretreatment on vecuronium and atracurium induced neuromuscular block. *Br J Anaesth* 1991; 66: 713-5.
- 4 *Woodworth GE, Sears DH, Grove TM, Ruff RH, Kosek PS, Katz RL.* The effect of cimetidine and ranitidine on the duration of action of succinylcholine. *Anesth Analg* 1989; 68: 295-7.
 - 5 *Law SC, Ramzan IM, Brandom BW, Cook DR.* Intravenous ranitidine antagonises intense atracurium-induced neuromuscular blockade in rats. *Anesth Analg* 1989; 69: 611-3.
 - 6 *Mishra Y, Ramzan I.* Interaction between succinylcholine and cimetidine in rats. *Can J Anaesth* 1992; 39: 370-4.
 - 7 *Kaka JS.* Rapid method for cimetidine and ranitidine determination in human and rat plasma by HPLC. *Journal of Liquid Chromatography* 1988; 11: 3447-56.
 - 8 *Holford NHG, Sheiner LB.* Understanding the dose-effect relationship: clinical application of pharmacokinetic-pharmacodynamic models. *Clin Pharmacokinet* 1981; 6: 429-53.
 - 9 *Hansen WE, Bertl S.* Inhibition of cholinesterases by ranitidine. (letter) *Lancet* 1983; 1: 235.
 - 10 *Garg DC, Weidler DJ, Eshelman FN.* Ranitidine bioavailability and kinetics in normal male subjects. *Clin Pharmacol Ther* 1983; 33: 445-52.