INTERACTION OF MIDAZOLAM WITH TWO NON-DEPOLARIZING NEUROMUSCULAR BLOCKING DRUGS IN THE RAT IN VIVO SCIATIC NERVE–TIBIALIS ANTERIOR MUSCLE PREPARATION

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Considerable controversy exists in the literature concerning the interaction between benzodiazepines and neuromuscular blocking drugs (see review by Haefely and colleagues (1981)). In this regard diazepam has been studied almost exclusively, and was shown not to potentiate non-depolarizing drugs in the majority of \textit{in vivo} investigations in rats and cats (Crankshaw and Raper, 1968; Dretchen, Ghoneim and Long, 1971; Webb and Bradshaw, 1973; McIndewar and Marshall, 1981). However, Vergano and co-workers (1969), using the isolated frog sciatic nerve–gastrocnemius muscle preparation, and Cheymol and associates (1967), using the rabbit sciatic nerve–tibialis anterior muscle preparation, demonstrated that diazepam did potentiate the effects of some non-depolarizing neuromuscular blocking drugs.

Like diazepam, most benzodiazepines used in clinical practice are poorly water-soluble and contain organic solvents which are not pharmacologically inert (Crankshaw and Raper, 1971). For example, the pure solvent of diazepam in Valium has been found to antagonize the response to non-depolarizing neuromuscular blocking drugs in animal \textit{in vivo} nerve–muscle preparations (Dretchen, Ghoneim and Long, 1971; Webb and Bradshaw, 1973).

Midazolam is a newer benzodiazepine derivative. Its hydrochloric acid is very stable in water buffered at pH < 4, and its pharmacological potency in the rat is similar to that of diazepam.

\textbf{SUMMARY}

Studies of the possible interactions between the water-soluble benzodiazepine, midazolam, and two non-depolarizing neuromuscular blocking drugs, vecuronium and tubocurarine, were performed in the rat \textit{in vivo} sciatic nerve–tibialis anterior muscle preparation. Midazolam 0.5 and 5 mg kg\(^{-1}\) caused 17\% and 34\% depression of the twitch height, respectively, once a steady-state blockade of the twitch height had been induced by vecuronium. The potentiation of the tubocurarine steady state blockade by midazolam 5 mg kg\(^{-1}\) was quantitatively equal to that of vecuronium, but was slower in onset. The buffer solvent of midazolam 5 mg kg\(^{-1}\) did not change the steady-state blockade of vecuronium. Midazolam 5 mg kg\(^{-1}\) caused a shift to the left of the cumulative dose–response curves of vecuronium (Pieri et al., 1981). This study investigated the effect of the i.v. administration of midazolam, in sedative doses, on the action of two non-depolarizing neuromuscular blocking drugs, vecuronium and tubocurarine, in the rat sciatic nerve–tibialis anterior muscle preparation.

\textbf{MATERIALS AND METHODS}

Wistar rats, weighing 250–430 g, were anaesthetized with pentobarbitone sodium 60 mg kg\(^{-1}\) i.p. Both jugular veins were cannulated with polyethylene catheters for drug administration. Arterial pressure (carotid artery) was recorded on a Grass polygraph. Tracheotomy was performed and ventilation with 50\% nitrous oxide in oxygen.
was controlled mechanically to achieve \( P_{\text{aCO}_2} \) 4–4.5 kPa. Arterial blood-gas tensions were monitored to ensure normoventilation. Rectal temperature and the temperature of the prepared muscle were measured and maintained between 37 and 38 °C by means of a thermostatically-controlled heating pad under the animal and a heating lamp on the muscle. The tendon of the left anterior tibialis muscle was freed, sectioned and connected to an FT03 force displacement transducer. The sciatic nerve was cut and the distal end stimulated by supramaximal rectangular (current) single stimuli of 0.2 ms duration at 0.1 Hz through a bipolar electrode. A resting tension of 20 g was applied to the muscle. The resulting twitch tension was recorded on the Grass polygraph. Anaesthesia was maintained by a continuous infusion of pentobarbitone sodium 9–18 mg kg\(^{-1}\) h\(^{-1}\).

Two investigations were performed: in the first the effect of midazolam on the twitch height was determined in the presence of steady-state neuromuscular blockade. In most of these studies vecuronium was administered i.v. by a constant infusion to achieve approximately 50% blockade of the original twitch height. In a few studies tubocurarine was administered similarly. In control studies \((n=5)\) the stability of the neuromuscular blockade produced by vecuronium was verified. When the steady-state depression of the twitch height (of about 50%) was constant for at least 15 min, the rate at which vecuronium was infused was kept constant for 45 min and the change of twitch height measured over this period. The stability of a tubocurarine-induced steady state neuromuscular block was assessed similarly \((n=5)\).

In six rats the buffer solvent of midazolam in Dormicum (NaCl 5 mg, benzylalcohol 10 μl, 1N HCl 28 μl, 1N NaOH to achieve pH 3.3 ± 0.1, water 1 ml) was injected i.v. once the steady-state blockade had been constant for at least 15 min. The amount of solvent used was equal to that present in midazolam 5 mg kg\(^{-1}\).

In six rats midazolam 0.5 mg kg\(^{-1}\) was administered i.v. as a bolus over 2 min once the blockade had been constant for 15 min. In 15 other rats, midazolam 5 mg kg\(^{-1}\) was administered similarly.

In six rats a constant degree of neuromuscular blockade was produced by an i.v. infusion of tubocurarine and the effect of midazolam 5 kg mg\(^{-1}\) measured.

In all these studies, the resultant percentage change in twitch height, from its steady-state value, was measured for 45 min from the injection of midazolam or solvent, while the infusion of the neuromuscular blocking drug was continued. Student's \( t \) test was used to assess the statistical significance of the resultant maximal changes in twitch height in comparison with the changes in control steady state. In the 15 rats receiving midazolam 5 mg kg\(^{-1}\), mean arterial pressures were monitored at 2-min intervals and the changes were analysed statistically by Student’s \( t \) test for paired data.

In the second group of investigations, cumulative dose–response curves for vecuronium were established 15 min after the administration of midazolam 5 mg kg\(^{-1}\) i.v. in eight rats and compared with control dose–response curves in eight other rats anaesthetized similarly, but not receiving midazolam. Incremental doses of vecuronium were given when the previous dose had achieved its maximal effect; that is, when two consecutive twitches of equal height were observed. This was always carried out with two investigators to co-ordinate the observation of twitches and the injection of vecuronium. The dose–response curves were analysed using the model in which the percentage blockade is described as a normal probability integral function of the dose of neuromuscular blocker in a linear scale (Robertson et al., 1983). The mean \( ED_{50} \) (dose expected to give 50% block of the twitch height) and \( \sigma/ED_{50} \) (slope of the curve) were determined for both groups of dose–response curves (control–vecuronium and midazolam–vecuronium).

Student’s \( t \) test for unpaired data was used for statistical analysis and \( P < 0.05 \) was considered as the level of significance.

RESULTS

**Steady state studies**

Once a steady-state depression of the twitch height of at least 15 min duration had been achieved with vecuronium, the mean deviation of the twitch height during the next 45 min of infusion was maximally +4.1% (SEM ± 2.6) from the twitch height at constant blockade. During control steady-state blockade with tubocurarine the mean deviation varied between +1.75% (4.5) at 25 min and −5.2% (2.5) at 60 min. This proved that the model was stable. Tracings of single control experiments of steady state neuromuscular block-
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Fig. 1. Representative records of single control studies in which steady-state neuromuscular blockade was induced and maintained with constant infusions of vecuronium or tubocurarine.

Fig. 2. Effect of midazolam on a steady-state blockade of the twitch height of vecuronium or tubocurarine in the rat in vivo sciatic nerve–tibialis anterior muscle preparation. The mean changes in percentage from the twitch height at steady-state blockade after the injection of midazolam i.v. (= time 0) are shown. SEM are shown only for the effect of midazolam 5 mg kg⁻¹ on a vecuronium-induced blockade, for reasons of clarity. □ = Midazolam 0.5 mg kg⁻¹ on a vecuronium blockade (n = 6); ◦ = midazolam 5 mg kg⁻¹ on a vecuronium blockade (n = 15); ○ = midazolam 5 mg kg⁻¹ on a tubocurarine blockade (n = 6).

When the solvent of midazolam was tested, the resultant changes in twitch height were negligible (mean maximal change = +2.1%) in a 30-min period. With midazolam 0.5 mg kg⁻¹ there was an additional depression of the twitch height of 17% (3) from the value reached at steady-state blockade with vecuronium. This potentiation was maximal at 15 min after injection and was significant (P = 0.004). With midazolam 5 mg kg⁻¹ a 34% (4) additional depression from the twitch height at steady state blockade with vecuronium occurred at 15 min after injection and returned to 11% (5) at 45 min (fig. 2). The change of the twitch height at 15 min was significant when compared with the control as well as with the change after the administration of the solvent (P < 0.001). Midazolam 5 mg kg⁻¹ caused a significantly higher potentiation of the vecuronium-induced steady-state blockade than did midazolam 0.5 mg kg⁻¹. When midazolam 5 mg kg⁻¹ was administered in
FIG. 3. Representative record showing steady-state blockade with vecuronium. At the arrow midazolam 5 mg kg\(^{-1}\) was injected i.v. while the infusion of vecuronium was continued. Note that the depression of the twitch height after midazolam showed a biphasic pattern.

Cumulative dose–response curves

The dose–response curve of vecuronium was shifted to the left in association with midazolam 5 mg kg\(^{-1}\), which dose decreased the ED\(_{50}\) of vecuronium significantly from 297 (36) to 192 (28) µg kg\(^{-1}\). The slope of the curve was not altered significantly (fig. 4).

DISCUSSION

In rats, midazolam and diazepam are almost equipotent in most pharmacological tests. The i.v. dose of midazolam which causes sedation and "muscle relaxation" in the rat is 5 mg kg\(^{-1}\) (Pieri et al., 1981). This is in agreement with i.v. sedative doses of diazepam in the rat reported by others (Ducksook and Dobkin, 1973; Carlsson et al., 1976).

The vecuronium- and tubocurarine-induced steady-state depression of the twitch height was stable over a period of at least 30–45 min. A
constant infusion of a non-depolarizing neuromuscular blocking drug is useful in the rat sciatic nerve–tibialis muscle preparation for studies on interactions, as has been demonstrated by other investigators (Miller et al., 1978; Krieg et al., 1980). This is especially true for vecuronium, since it does not show evidence of cumulation, either in effect or in plasma concentration (Agoston et al., 1980).

Our results show that neuromuscular blockade produced by vecuronium was potentiated by sedative doses of midazolam in the rat. This potentiation was attributable to midazolam and not to the solvent. The potentiation was dose-dependent and time-related. With tubocurarine the time-course of the potentiation was different: slower in onset and prolonged in duration. However, we cannot exclude the possibility that this prolonged potentiation of tubocurarine was caused in part by some cumulation of tubocurarine.

Several mechanisms could contribute to the interactions between neuromuscular blocking agents and other drugs (Waud, 1981). Since the dose–response curve of vecuronium was shifted to the left and did not deviate significantly from parallelism after midazolam 5 mg kg\(^{-1}\), this provides some evidence for an interaction at the level of the neuromuscular junction or the muscle, or both. However, other mechanisms of interaction (protein binding, elimination kinetics and haemodynamic changes) must be studied further. Since the sciatic nerve was cut, spinal reflex mechanisms were excluded. The different time-courses of the interactions of midazolam with vecuronium and tubocurarine may be related to differences in the activity of the two non-depolarizing blocking drugs at different receptors in the neuromuscular junction. The response of the arterial pressure to midazolam suggests that this is not the main mechanism of interaction, although benzodiazepines may change the local distribution of blood to muscles without any significant change in arterial pressure.

The fact that the solvent of midazolam had no effect has ensured a reliable assessment of the interaction of midazolam per se with these neuromuscular blocking drugs. The question remains whether midazolam behaves differently from other benzodiazepines in regard to the peripheral interaction with non-depolarizing neuromuscular blocking drugs. This can only be answered in interaction studies with different benzodiazepines and by thoroughly assessing the effects of other solvents which can markedly influence the effects of the benzodiazepines themselves (Crankshaw and Raper, 1971; Dretchen, Ghoneim and Long, 1971; Webb and Bradshaw, 1973). The solvent of commercial diazepam (Valium) and some other injectable benzodiazepines may well obscure possible potentiating effects of the active substances. Studies in the rat in vitro phrenic nerve–hemidiaphragm preparation showed differences in the response of the twitch contraction to several benzodiazepines (Driessen et al., 1984).

In the present study, considerable variation was observed in the potentiating effect of midazolam on non-depolarizing neuromuscular blocking drugs. Variability of action is common with several benzodiazepines: File (1982) found profound variability in some responses to benzodiazepines in rats without pharmacokinetic differences. In practice, variability means that in some instances unexpectedly marked potentiation may occur, and this emphasizes the need for further investigations of this kind of interaction. It may be worth noting in the light of our studies that Feldman and Crawley (1970) observed prolonged postoperative muscle weakness after combined use of diazepam and tubocurarine in patients.

The neuromuscular function measured with the methods of evoked isometric twitch contractions has a "large margin of safety" (Paton and Waud, 1967). When this margin is decreased by different conditions (myasthenia gravis; hypokalaemia; concomitant use of some antibiotic drugs; liver or kidney dysfunction) vigilance and monitoring are necessary in the use of neuromuscular blocking drugs in combination with benzodiazepines.

In conclusion, the significant potentiation of vecuronium and tubocurarine by midazolam 0.5 and 5 mg kg\(^{-1}\) justifies further studies to compare the effects of other injectable formulations of benzodiazepines and their solvents. Since extrapolation of the results to man is difficult, human studies with clinically-used benzodiazepines should be undertaken.

ACKNOWLEDGEMENTS
The authors wish to thank Francien van der Pol and Marjon van de Broek for their technical assistance in the performance of the studies.
REFERENCES


