THE PHARMACOLOGY OF AH 8165: A RAPID-ACTING, SHORT-LASTING, COMPETITIVE NEUROMUSCULAR BLOCKING DRUG

R. T. BRITTAIN AND M. B. TYERS

SUMMARY

1,1'Azobis[3-methyl-2-phenyl-1H-imidazo(1,2-a)pyridinium] dibromide, AH 8165, produced a competitive neuromuscular block in the mouse, chick, cat, dog and monkey. The paralysis was characterized by a very rapid onset of action and, in all species except the monkey, by a short duration of action comparable to that of suxamethonium. In the monkey, the effect of AH 8165 was more persistent but was shorter than that obtained with gallamine or pancuronium. The neuromuscular block induced by AH 8165 was rapidly and completely reversed by neostigmine. Fully effective neuromuscular blocking doses of AH 8165 produced no adverse effects on blood pressure or the electrocardiogram; at much higher doses AH 8165 caused a fall in blood pressure due to a ganglion blocking action. AH 8165 did not release histamine.

It is considered that AH 8165 has advantages over existing neuromuscular blocking agents and merits detailed study in clinical trial.

In present-day anaesthetic practice, the depolarizing muscle relaxant suxamethonium is used extensively because of its rapid onset and brevity of action. However, suxamethonium has several inherent disadvantages. For example, there is a high incidence of postoperative muscle pain following the use of this muscle relaxant (Churchill-Davidson, 1954). In addition, suxamethonium can induce hyperkalaemia and cardiac irregularities and also occasional prolonged apnoea in patients deficient in serum pseudocholinesterase (Evans et al., 1952, 1953). Competitive muscle relaxants, such as (+)-tubocurarine, gallamine and pancuronium do not have these disadvantages but their actions are more persistent than those of suxamethonium. Recently a series of azobisarylimidazo-[1,2-a]-pyridinium dihalides has been shown to possess potent and short-lasting competitive muscle relaxant properties (Bolger et al., 1972); of these the most interesting compound was 1,1'azobis[3-methyl-2-phenyl-1H-imidazo(1,2-a)pyridinium] dibromide, AH 8165 (Brittain and Tyers, 1972). Initial clinical trial results with AH 8165 are encouraging (Simpson et al., 1972; Blogg, 1973; Coleman et al., 1973) and the trials are being extended. AH 8165 has been studied in detail in many animal species and the results obtained are reported here.

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drug together with 95% fiducial limits were calculated by the method of Litchfield and Wilcoxon (1949).

**Actions in chicks.**
Paralysing activity was determined in 7-day-old chicks. Groups of 6 chicks were used for each dose level. The ED50 (paralysing activity) for each drug, together with 95% fiducial limits was calculated by the method of Litchfield and Wilcoxon (1949).

**Neuromuscular blocking actions in anaesthetized cats, dogs and monkeys.**
Cats (1.8–3.5 kg) were anaesthetized with chloralose (80 mg/kg intravenously) following induction with 3.5% halothane in a nitrous oxide/oxygen mixture (3:1). The tibialis anterior muscle tendon of the left hind leg was separated from its insertion on to the medial side of the foot by cutting away a small piece of the bone forming part of the first metatarsal. A length of braided nylon thread was tied around the tendon and was later attached to a Statham 32-oz strain gauge to record muscle tension. The sciatic nerve was located between the adductor femoris and biceps femoris muscles from the ventral surface of the thigh and crushed just centrally to its division into the tibial and peroneal nerves. The tibial nerve was also crushed and a shielded bipolar platinum electrode was placed on the peroneal nerve. The wound edges were sutured with metal clips such that the electrode was secured in place. The lower leg was then fixed in an extended horizontal position using two metal clamps. One clamp had been modified such that two sharpened screws protruded into the jaws of the clamp and could be firmly pinned to the distal end of the femur. The other clamp was attached firmly to the foot so as not to interfere with the movement of the tendon. The tibialis anterior muscle was held at a resting tension of 40 g through the experiment. The peroneal nerve was stimulated at a frequency of 1.0 Hz with rectangular pulses of supramaximal voltage and 0.2 msec duration using a Tektronix Series 160 stimulator and type 2620 stimulus isolator. In some experiments the tibialis muscle was stimulated directly using a concentric stainless steel bipolar electrode inserted into the belly of the muscle; antidromic action potentials were recorded from the sciatic nerve using a Tektronix type 122 low-level pre-amplifier and RM565 dual-beam oscilloscope and polaroid camera. In other experiments the tibialis muscle surface potential was recorded following the method of Hughes (1972). Drugs were given through a cannula inserted in a jugular vein.

Similar experiments were also carried out using beagle dogs (7.4–10.5 kg) and cynomolgus monkeys (1.8–2.7 kg). Both dogs and monkeys were anaesthetized with pentobarbitone (20–30 mg/kg intravenously).

**Neuromuscular blocking action in isolated skeletal muscle preparations.**

**Rat phrenic nerve-diaphragm preparation.** Neuromuscular blocking potency was determined following the method of Büllbring (1946). The diaphragm was stimulated indirectly via the phrenic nerve using trains of rectangular pulses (45 Hz for 0.2 sec and 0.2 msec pulse width) of supramaximal intensity given once every 15 sec. Muscle twitches were recorded using a Statham 2-oz strain gauge. Cumulative dose-response curves for the effects of drugs to block neuromuscular transmission were determined and the EC50 values (concentration to cause 50% inhibition of muscle twitches) were calculated.

**Chick biventer-cervicis muscle preparation.** Neuromuscular blocking activity was determined using the method of Ginsberg and Warriner (1960). The “nerve-tendon” was stimulated using trains of rectangular pulses (35 Hz for 0.2 sec and pulse width 0.2 msec) at supramaximal intensity once every 15 sec. Muscle twitches were recorded using a Statham 2-oz strain gauge. Neuromuscular blocking potencies of drugs were calculated as described above.

**Actions on the cardiovascular system in anaesthetized cats, dogs and monkeys.**
The effects of AH 8165 on the cardiovascular system were investigated in cats anaesthetized with chloralose (80 mg/kg intravenously), dogs anaesthetized with thiopentone (25 mg/kg intravenously) and barbitone (25 mg/kg i.p.) and in monkeys anaesthetized with pentobarbitone (25 mg/kg intravenously). Femoral or carotid blood pressure, pulse rate and electrocardiogram (lead II) were recorded in all experiments. The effects of single intravenous doses
of AH 8165 and prolonged slow intravenous infusions on responses to bilateral occlusion of the common carotid arteries, to injected noradrenaline, dimethylphenylpiperazinium iodide (DMPP) and isoprenaline were determined. The effects of drugs on the responses to peripheral vagal nerve stimulation on blood pressure, heart rate and gastric motility were also recorded.

**Effects on ganglionic transmission.**

In the chloralose anaesthetized cat responses of the nictitating membrane to periodic preganglionic stimulation for 6 sec of the ascending cervical sympathetic nerve (16 Hz, 0.5 msec pulse width, supramaximal intensity) were measured using a 2-oz strain gauge transducer and recorded on a Devices M4 recorder. Ganglion blocking activity was also investigated in vitro on responses of the guineapig vas deferens preparation to pre- and postganglionic stimulation (Ohlin and Strömblad, 1963).

**Histamine release.**

Histamine release by AH 8165 was investigated in two species. In guineapigs the bronchoconstrictor test of Konzett and Rössler (1940) was used as well as the formation of a weal following intradermal injection. In cats, the delayed depressor response described by Collier and Macauley (1952) and the effects of injection of mepyramine were studied.

**Acute toxicity.**

AH 8165 was infused intravenously in artificially ventilated anaesthetized cats. The infusion was increased to determine the maximum tolerated dose. LD50 values were also determined in albino mice (18–25 g) after intravenous injection. Five mice were used in each group and results were calculated using the method of Litchfield and Wilcoxon (1949).

**RESULTS**

**Actions in mice.**

AH 8165, 0.05–0.2 mg/kg intravenously, in mice caused a flaccid paralysis which was very rapid in onset (<15 sec) and lasted from 1 to 4 min. The paralysis was not preceded or accompanied by muscle fasciculations. The paralysing activities (ED50) and acute toxicities (LD50) of AH 8165, suxamethonium, gallamine and pancuronium are summarized in table I.

**Actions in chicks.**

In 7-day-old chicks AH 8165, 0.03–0.2 mg/kg intravenously, caused a flaccid paralysis which was rapid in onset and short-lasting (0.5–2 min). The ED50 value (95% fiducial limits) was 0.055 (0.043–0.069) mg/kg.

**Neuromuscular blocking actions in anaesthetized cats, dogs and monkeys.**

In chloralose anaesthetized cats, AH 8165, 0.2 mg/kg intravenously, depressed maximal twitches of the tibialis muscle by 60.4±3.7% following stimulation of the peroneal nerve at a frequency of 1 Hz. The development of block was very rapid (<20 sec) and lasted from 1 to 2½ min. The neuromuscular blocking actions of AH 8165, gallamine, pancuronium and suxamethonium in the anaesthetized cat are illustrated in figure 1. In those preparations in which both tibialis anterior muscles were stimulated but at different frequencies, AH 8165 selectively inhibited the muscle stimulated at the higher frequency. For example, 0.2 mg/kg intravenously depressed muscle twitches elicited at 1 Hz by 74.0±9.2% without affecting twitches of the muscle stimulated at 0.1 Hz.

Details of potency and time course of neuromuscular block induced by AH 8165 in the anaesthetized cat, dog and monkey are summarized in table II.

In the anaesthetized cat the "cumulation time", defined as the shortest time interval required for repeated doses to produce equal effects, was 9 min for AH 8165 (fig. 2). This was 2–3 times shorter than that reported for suxamethonium (Busfield et al., 1968). A slow intravenous infusion of AH 8165, 60 (μg/kg)/min, maintained near complete neuromuscular block which could be rapidly varied by altering the rate of infusion. When the infusion was stopped after 2–3 hours the muscle twitch tension returned to pre-drug levels in 5–7 min.

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**Table I. Paralysing activities (ED50) and acute toxicities (LD50) of AH 8165, suxamethonium, gallamine and pancuronium, following intravenous injection to conscious mice.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Paralysing activity (ED50) (95% fid. limits)</th>
<th>Acute toxicity (LD50) (95% fid. limits)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH 8165</td>
<td>0.098 (0.08-0.12)</td>
<td>0.19 (0.16-0.23)</td>
</tr>
<tr>
<td>Suxamethonium</td>
<td>0.10 (0.07-0.14)</td>
<td>0.80 (0.62-1.00)</td>
</tr>
<tr>
<td>Gallamine</td>
<td>0.86 (0.72-1.01)</td>
<td>1.70 (1.50-2.20)</td>
</tr>
<tr>
<td>Pancuronium</td>
<td>0.0072 (0.0061-0.0096)</td>
<td>0.014 (0.011-0.018)</td>
</tr>
</tbody>
</table>
**Fig. 1.** Comparative effects of AH 8165, gallamine, pancuronium and suxamethonium on tibialis muscle twitches (1 Hz), blood pressure and heart rate in the chloralose anaesthetized cat.

**TABLE II.** Neuromuscular blocking potencies and time courses of action of AH 8165 in the anaesthetized cat, dog and monkey following intravenous injection.

<table>
<thead>
<tr>
<th>Species (number of animals)</th>
<th>Dose (mg/kg i.v.)</th>
<th>Time from injection to first detectable action (sec)</th>
<th>Time for detectable action to maximum effect (sec)</th>
<th>Mean block (%)</th>
<th>Recovery time (min)</th>
<th>50% (range)</th>
<th>90% (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat (10)</td>
<td>0.1</td>
<td>(7.0–10.0)</td>
<td>(6.0–15.0)</td>
<td>12.73</td>
<td>0.62 (0.26–1.00)</td>
<td>0.87</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>(4.0–7.0)</td>
<td>(8.0–16.0)</td>
<td>60.40</td>
<td>1.03 (0.26–1.65)</td>
<td>1.73</td>
<td>2.56</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>(4.0–6.0)</td>
<td>(7.0–18.0)</td>
<td>93.00</td>
<td>1.77 (1.11–2.70)</td>
<td>1.53–3.80</td>
<td>2.56</td>
</tr>
<tr>
<td>Dog (3)</td>
<td>0.05</td>
<td>(12.0–17.0)</td>
<td>(24.0–34.0)</td>
<td>12.30</td>
<td>0.92 (0.72–1.10)</td>
<td>1.10–1.86</td>
<td>2.96</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>(14.0)</td>
<td>(22.5)</td>
<td>60.20</td>
<td>1.88 (1.55–2.20)</td>
<td>2.58–3.40</td>
<td>6.56</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>(12.0–16.0)</td>
<td>(18.0–27.0)</td>
<td>70.00</td>
<td>3.91 (2.84–6.00)</td>
<td>5.10–7.40</td>
<td>14.6</td>
</tr>
<tr>
<td>Monkey (3)</td>
<td>0.05</td>
<td>(10.0–14.0)</td>
<td>(15.0–19.0)</td>
<td>28.30</td>
<td>2.06 (2.04–2.62)</td>
<td>4.25</td>
<td>6.80</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>(12.0–21.0)</td>
<td>(18.0–26.0)</td>
<td>83.50</td>
<td>4.76 (1.10–2.62)</td>
<td>2.0–5.71</td>
<td>8.80</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>(13.0–15.0)</td>
<td>(14.0–18.0)</td>
<td>80.0–87.9</td>
<td>7.03 (3.62–5.94)</td>
<td>6.66–9.20</td>
<td>14.6</td>
</tr>
</tbody>
</table>

Values quoted in table are mean responses (range).

**Site and mechanism of action.**

Investigations into the site and mechanism of neuromuscular blocking action of AH 8165 were carried out mostly in the anaesthetized cat; a few experiments were repeated in the anaesthetized dog and monkey.

The muscle paralysis induced by AH 8165 was caused by an action at the neuromuscular junction because (a) responses of the muscle to indirect stimulation were abolished following AH 8165 administration whilst responses to direct stimulation were unaffected, (b) gross muscle action potentials were abolished during AH 8165 block whereas the antidromic action potentials recorded from the sciatic nerve were unaffected, and (c) during partial block with AH 8165 the muscle responses to close-arterial injections of acetylcholine (5 µg) were completely abolished.
The neuromuscular block produced by AH 8165 was never preceded by a potentiation of the muscle twitch response nor did the drug cause muscle fasciculations. The block could be readily reversed with anticholinesterase drugs; for example, administration of neostigmine, 50 µg/kg intravenously, quickly and completely reversed the block. In addition, suxamethonium, 50 µg/kg intravenously, and other depolarizing agents reversed the induced neuromuscular block. Competitive neuromuscular blocking drugs administered concurrently with AH 8165 had additive blocking effects. Tetanic stimulation of the peroneal nerve during AH 8165 block produced a poorly sustained response and a post-tetanic potentiation of muscle twitches (fig. 2). Recordings of muscle surface potential were unchanged during AH 8165 neuromuscular block. All these results indicated that the mechanism of the neuromuscular block produced by AH 8165 was competitive.

Neuromuscular blocking actions on isolated skeletal muscle preparations.

**Rat phrenic nerve-diaphragm.** AH 8165 4 µg/ml abolished muscle twitches elicited indirectly once every 15 sec on this preparation. Onset of neuromuscular block was rapid and, on washing the tissue, muscle twitches returned rapidly to pre-drug levels. The neuromuscular blocking potencies of AH 8165, gallamine, pancuronium and suxamethonium are compared in table III.

**Chick biventer cervicis muscle.** On the chick biventer cervicis muscle preparation AH 8165 2 µg/ml abolished maximal twitches elicited once every 15 sec. Onset of block was rapid and there was no change in resting muscle tension. On washing the tissue, muscle twitches returned rapidly to pre-drug levels. The neuromuscular blocking potencies of AH 8165, gallamine, pancuronium and suxamethonium are also given in table III.

**Actions on the cardiovascular system in anaesthetized cats, dogs and monkeys.**

In artificially ventilated, anaesthetized cats, dogs and monkeys neuromuscular blocking doses of AH 8165, 0.1-0.8 mg/kg intravenously, had no effect on blood pressure or e.g. but slight increases in pulse rate (+12±8/min) were occasionally recorded. This latter effect was most common in chloralose anaesthetized cats. It was interesting to find that AH 8165 0.2-0.8 mg/kg prevented or reversed cardiac arrhythmias induced by intubation or adrena-

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**Table III. Neuromuscular blocking actions of AH 8165 gallamine, pancuronium and suxamethonium on isolated nerve-muscle preparations of the rat and chick.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>EC50: concentrations to cause 50% inhibition of muscle twitches (µg/ml, 95% fiducial limits)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat phrenic nerve-diaphragm preparation</td>
<td>Chick biventer cervicis muscle preparation</td>
</tr>
<tr>
<td>AH 8165</td>
<td>0.68 (0.28-1.6)</td>
</tr>
<tr>
<td>Gallamine</td>
<td>16.5 (14.9-18.3)</td>
</tr>
<tr>
<td>Pancuronium</td>
<td>0.09 (0.052-0.13)</td>
</tr>
<tr>
<td>Suxamethonium</td>
<td>1.25 (0.96-1.53)</td>
</tr>
</tbody>
</table>
line in thiopentone anaesthetized dogs; gallamine 0.5 mg/kg and (+)-tubocurarine 0.3 mg/kg had similar effects. AH 8165 0.5 mg/kg selectively inhibited the depressor effects of peripheral vagal nerve stimulation on heart rate and blood pressure without affecting increases in gastric motility.

Higher doses of AH 8165, 1.0–5.0 mg/kg, caused dose-dependent falls in blood pressure (5–50 mm Hg) accompanied by small changes in pulse rate. The depressor responses were not affected by prior administration of the antihistaminic agent mepyramine, 0.5 mg/kg.

**Effects on ganglonic transmission.** The depressor responses induced by large doses of AH 8165 on the blood pressure of anaesthetized animals can be attributed to ganglion blockade. In the anaesthetized cat, AH 8165 1.0–5.0 mg/kg reduced contractions of the nictitating membrane induced by periodic preganglonic nerve stimulation. In addition, in the anaesthetized dog the same doses of AH 8165 reduced vasopressor responses to DMPP (1 μg/kg).

On the isolated guineapig vas deferens preparation, AH 8165 30 μg/ml inhibited contractions of the muscle to preganglonic nerve stimulation without affecting the contractions to postganglonic stimulation.

Excessive doses of AH 8165 (e.g. 100 mg/kg/min for 10 min intravenous infusion) were well tolerated by artificially ventilated anaesthetized cats. Following this dose of AH 8165 the blood pressure was markedly reduced and bradycardia ensued; T-wave inversion was the only change recorded in the electrocardiogram. Normal blood pressure and heart rate were restored after 6–8 hr.

**Histamine release.** In conscious guineapigs, AH 8165 20–200 μg intradermally did not cause weals; nor, in the anaesthetized animals, did AH 8165 0.1–10.0 mg/kg intravenously, affect resting bronchial resistance. Clearly, AH 8165 does not release histamine in this species.

**DISCUSSION**

AH 8165 is a potent, competitive neuromuscular blocking agent in the mouse, cat, dog and monkey. It has an extremely rapid onset of action and is considerably shorter-acting than either pancuronium or (+)-tubocurarine. Effective neuromuscular blocking doses of AH 8165 have no adverse effects on the cardiovascular system. These results indicated that AH 8165 might possess desirable clinical properties and it was, therefore, submitted for clinical trial following appropriate toxicological studies.

In man, AH 8165 0.25–1.0 mg/kg caused muscle paralysis which was suitable for rapid intubation and subsequent surgery (Simpson et al., 1972). The development of paralysis was consistently more rapid than that obtained with suxamethonium and was not accompanied by muscle fasciculations. However, the duration of action in man was longer than in animals, being similar to that of pancuronium. This result most resembled its effect in the anaesthetized monkey than in other species. The importance of using the monkey when extrapolating recovery times of neuromuscular blocking agents from animals to man has been reported previously (Mushin and Mapleson, 1964; Bamford et al., 1967; Norman and Katz, 1971; Hughes, 1972).

The concept that interquaternary distance in bisquaternary nitrogen compounds is a critical factor for neuromuscular blocking efficacy (Paton and Zaimis, 1948, 1949; Barlow and Ing, 1948a,b) has been criticized on several occasions (Loewe and Harvey, 1952; Waser, 1959; Alaudin et al., 1965; Lonsdale et al., 1965; Bamford et al., 1967; Busfield et al., 1968; Everett, Lowe and Wilkinson, 1970). The interquaternary distance for AH 8165 is 7.48Å (Pointer, Wilford and Bishop, 1972) which is much less than the once commonly supposed optimum distance of 12.5Å for neuromuscular blocking activity. Indeed, according to earlier views AH 8165 would be expected to have a greater inhibitory effect at ganglia than at the motor endplate. Effective neuromuscular blocking doses of AH 8165 are not associated with ganglion blocking activity although the latter action can explain the hypotensive effects of AH 8165 seen after large doses.

A neuromuscular blocking agent as short-acting as suxamethonium but with a competitive mechanism of action would have advantages over the latter drug. According to current opinions a rapid onset of action is also important. Initial clinical trials showed AH 8165 to have a more rapid onset of action than suxamethonium and not to cause muscle fasciculations. AH 8165 may, therefore, be the preferred drug in emergency cases where surgery is necessary in obstetrics and unprepared patients (Simpson et al., 1972). In such cases suxamethonium sometimes causes an increase in intragastric pressure and regurgitation of gastric contents before intubation. Because of its rapid onset of action and freedom from cardiovascular effects at paralysing doses, AH 8165 has advantages over existing neuromuscular blocking agents and the clinical trials are being extended.
ACKNOWLEDGEMENTS

We are grateful to Mr. M. G. Dodds, Glaxo Research Laboratories, Fulmer, Bucks, for advice and assistance with the work carried out in anaesthetized monkeys. We acknowledge the technical assistance of Miss S. Victor and Mr. A. Cornish.

REFERENCES


THE SOCIETY OF ANAESTHETIC LABORATORY TECHNICIANS

The Autumn General and Scientific Meeting of the Society of Anaesthetic Laboratory Technicians will be held at the Magill Department of Anaesthetics, Westminster Hospital, Page Street, London SW1 2AP, on Friday and Saturday, October 12 and 13, 1973.

Submissions for papers should be addressed to Mr. R. Tennant at that address.