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PHARMACOLOGICAL AND CERTAIN CHEMICAL PROPERTIES OF AH 10407, AN UNUSUALLY SHORT-ACTING, COMPETITIVE NEUROMUSCULAR BLOCKING DRUG, AND SOME RELATED COMPOUNDS

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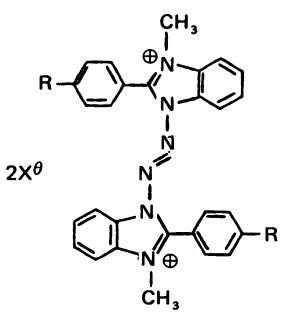
1 1,1′-Azobis[3-methyl-2-phenylbenzimidazolinium]dimethanesulphonate (AH 10407) has an ultra-short, competitive neuromuscular blocking action in the mouse, cat, dog, Cynamolgus monkey and cotton-eared marmoset.

2 AH 10407 is chemically unstable in bicarbonate-containing solutions and is degraded to inactive products. The half-life of AH 10407 *in vitro* in dog and human whole blood and in Krebs physiological solution is about 1.0 minute. In distilled water and in HCO_3^--deficient Krebs solution AH 10407 is much more stable. Base catalyzed degradation is shown to be the prime determinant of the duration of action of the drug.

3 Some pharmacological properties of AH 11244 and AH 11056, close analogues of AH 10407, are briefly described and the duration of their neuromuscular blocking actions rationalized by reference to their chemical stabilities.

Introduction

Many compounds expected to be easily susceptible to enzymatic degradation have been made in attempts to find a competitive neuromuscular blocking (NMB) drug with a short duration of action in man (Bruce, 1956; Haining, Johnston & Smith, 1960; Brittain, Collier & D'Arcy, 1961; Savarese, Nakamura & Kitz, 1970; Savarese, Ginsburg, Lee & Kitz, 1973) but only succinylcholine, a depolarizing agent, has proved satisfactory in this respect. An alternative, novel approach to the desired short-acting, competitive NMB drug in man was identified during the pharmacological and physico-chemical examination of 1,1′-azobis[3-methyl-2-phenylbenzimidazolinium] dimethanesulphonate (AH 10407; Blogg, Brittain, Simpson & Tyers, 1975; Tyers, 1975), one of a series of azobis-benzimidazolinium salts (Glover, Bishop & Rowbottom, 1973). AH 10407 (1a) is a competitive, neuromuscular blocking drug with an ultra-short duration of action in animals and in man. This paper describes the detailed pharmacology of AH 10407 in laboratory animals, including primates, and describes experiments undertaken to account for its brief action. A preliminary report on AH 10407 has been presented to the British Pharmacological Society (Blogg *et al.*, 1975). Limited pharmacological results are also presented on AH 11244 [1b] and AH 11056 [1c] which are close analogues of 10407.



1a. AH 10407 R=H
1b. AH 11244 R=CH_3
1c. AH 11056 R=OCH_3

Methods

Paralysing action in chicks

The paralysing activity of intravenous AH 10407 was determined in 7-day-old chicks. Groups of 6 chicks were used for each dose-level. The ED_{50} (paralysing

activity) for AH10407 was calculated by the method of Litchfield & Wilcoxen (1949).

Neuromuscular blocking actions in anaesthetized cats, dogs and monkeys

Cats (1.7–3.2 kg) were anaesthetized with chloralose (75 mg/kg intravenously) following induction with a mixture of halothane (3%) in nitrous oxide and oxygen (3:1). The tibialis anterior muscle was prepared for indirect stimulation via the peroneal branch of the sciatic nerve by the method described previously (Brittain & Tyers, 1973). The peroneal nerve was stimulated at a frequency of 1 Hz with rectangular pulses of supramaximal voltage and 0.2 ms duration. Tibialis anterior muscle twitches were recorded on an Elema-Schonander Mingograf recorder. The rate of onset, the intensity, the rate of recovery and the duration of the neuromuscular blocking actions of AH 10407 were determined from the following measurements of tibialis anterior muscle twitches; (a) the time from intravenous injection to the first reduction in twitch tension; (b) the time from the first reduction in twitch tension to maximum neuromuscular block; (c) the maximum percentage inhibition of muscle twitches; (d) the time from the first reduction to 50% recovery of the twitch height; (e) the time from first injection to 90% recovery of the twitch height. In some experiments the tibialis anterior muscle was stimulated directly by inserting a concentric, bipolar, stainless-steel electrode into the belly of the muscle. Close-arterial injections of drugs to the tibialis anterior muscle were carried out via the cannulated suralis branch of the popliteal artery; the femoral artery was occluded during close-arterial injections. Rectal temperature was monitored throughout. Where relevant, tibialis anterior muscle temperatures were measured with thermocouple probes inserted deep into the belly of the muscle.

Similar experiments were carried out in Beagle dogs (7.9–8.5 kg) Cynamolgus monkeys (1.6–2.3 kg) and cotton-eared marmosets (0.5–0.7 kg). The dogs and primates were anaesthetized with pentobarbitone (25–35 mg/kg i.v.) to effect and maintain surgical anaesthesia.

Neuromuscular blocking actions on the isolated phrenic nerve-diaphragm preparation of the rat

Neuromuscular blocking activity was determined on the isolated phrenic nerve-diaphragm preparation of the rat according to the method described by Brittain & Tyers (1973). The diaphragm was stimulated indirectly with trains of pulses (45 Hz for 0.2 s and 0.2 ms pulse width) of supramaximal intensity given once every 15 seconds. Neuromuscular blocking activities (EC_{50}) were calculated from cumulative dose-response curves.

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

The effects of single doses or continuous intravenous infusions of AH 10407 on the cardiovascular system were investigated in the anaesthetized cats, dogs, monkeys and marmosets used in the neuromuscular studies. The carotid blood pressure, pulse rate and the electrocardiogram (lead II) were recorded in all experiments. In the anaesthetized cat the effects of AH 10407 on the blood pressure and pulse rate responses induced by peripheral vagal nerve stimulation were also recorded.

Actions on ganglionic transmission

In cats anaesthetized with chloralose the effects of AH 10407 on contractions of the nictitating membrane to periodic, preganglionic stimulation for 6 s of the ascending cervical sympathetic nerve (6 Hz, 0.5 ms pulse width, supramaximal intensity) were measured with a 2 oz strain guage transducer and recorded on the chart recorder. In dogs anaesthetized with pentobarbitone the effects of AH 10407 on vasopressor responses to injected dimethylphenylpiperazinium iodide (DMPP) were also determined.

Histamine release

The histamine-releasing activity of AH 10407 was investigated in two species. In guinea-pigs anaesthetized with urethane the bronchoconstrictor test described by Dixon & Brodie (1903) was used. In anaesthetized cats the effects of mepyramine on the delayed vasodepressor response described by Collier & Macauley (1952) were studied.

Chemical stability

In early experiments (Tyers, 1975) it was found that the neuromuscular blocking action of AH 10407 on the rat isolated phrenic nerve-diaphragm preparation waned during 2 min continuous exposure to drug-containing Krebs solution. This result, together with the finding of Glover *et al.* (1973) that AH 10407 and some of its analogues were unstable in water in the presence of nucleophiles such as OH^- or HCO_3^- led us to a more systematic investigation of the stability of AH 10407 under a variety of conditions because base-catalyzed inactivation in blood seemed a possible determinant of its duration of action. Solutions of the drug in distilled water, aqueous solution adjusted to pH 1, 3 or 6 with hydrochloric acid, whole blood (dog and human), normal Krebs solution, Krebs solution without sodium bicarbonate and Krebs solution containing an equivalent amount of sodium acetate instead of bicarbonate were assayed after various periods of incubation at 24–40°C on the rat isolated phrenic

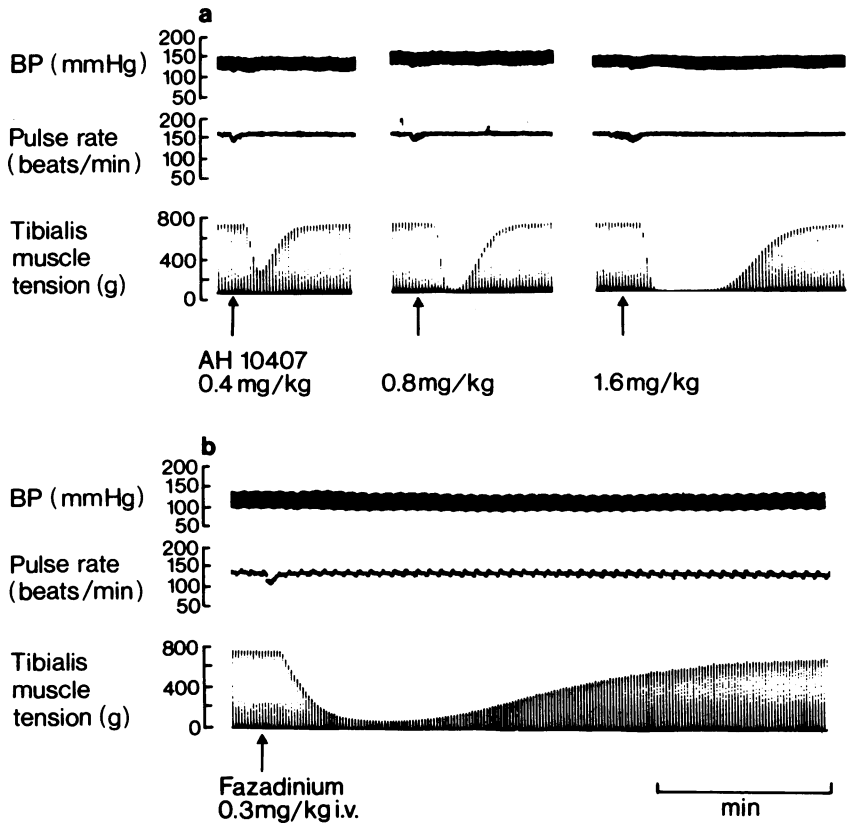


Figure 1 The effects of intravenous doses of (a) AH 10407 and (b) fazadinium dibromide (AH 8165D) on tibialis anterior muscle twitches stimulated indirectly at a frequency of 1 Hz, on arterial blood pressure (BP) and pulse rate in the cat anaesthetized with chloralose.

nerve-diaphragm preparation with the freshly prepared aqueous drug solution as standard.

Drugs and solutions

The following drugs were used:1,1′-azobis[3-methyl-2-phenylbenzimidazolinium] dimethanesulphonate (AH 10407); 1,1′-azobis[3-methyl-2-*p*-tolyl-benzimidazolinium]dibromide (AH 11244); 1,1′-azobis[3-methyl-2-*p*-methoxyphenyl-benzimidazolinium]dibromide (AH 11056); fazadinium dibromide (AH 8165, Allen & Hanburys Research Ltd.); (+)-tubocurarine chloride (Burroughs Wellcome); neostigmine methylsulphate (Roche Products Ltd.); atropine sulphate (Macfarlane Smith Ltd.); acetylcholine chloride (Sigma); dimethylphenylpiperazinium iodide. Drugs were dissolved and diluted in 0.9% w/v NaCl solution (saline). All doses and concentrations refer to the free bases. In anaesthetized preparations all drugs were given intravenously unless stated otherwise.

Results

Paralysing action in chicks

In conscious, 7-day-old chicks AH 10407, 0.8 mg/kg intravenously, caused a flaccid paralysis which lasted for 25 seconds. The ED_{50} (paralysing activity) values for AH 10407 and (+)-tubocurarine were 0.42 (0.28–0.60) mg/kg and 0.045 (0.035–0.05) mg/kg respectively.

Neuromuscular blocking actions in anaesthetized cats, dogs, Cynamolgus monkeys and marmosets

In the anaesthetized cat, AH 10407 0.5–2.0 mg/kg intravenously, depressed tibialis anterior muscle twitches (frequency, 1 Hz) by 14.3–100%. At these doses the neuromuscular block lasted from 6–50 seconds. The onset of block induced by AH 10407 was very rapid, the maximal effects being reached within 10–15 s after injection. The neuromuscular

blocking actions of AH 10407 and fazadinium in the cat are compared in Figure 1. AH 10407 had similar effects in the other species studied (Table 1). The species variation in sensitivity to AH 10407 was slight. The onset times of block were similar in the cat and Cynamolgus monkey, but were slightly longer in the dog and marmoset. AH 10407 was longer acting in the marmoset than in the cat, dog or Cynamolgus monkey, but even in this species, which has been found to resemble man closely regarding the duration of action of some competitive NMB drugs (Tyers, 1975), near-complete neuromuscular block lasted for only 3 minutes. In the anaesthetized cat the 'cumulation time' for repeated submaximal doses of AH 10407 was 1.5 min, indicating that full recovery of neuromuscular transmission was very rapid.

Intravenous infusions of AH 10407, 0.45 mg kg^{-1} min^{-1}, in the anaesthetized cat and Cynamolgus monkey caused 80–95% inhibition of twitches of the tibialis anterior muscle. When the infusions were terminated after 30 min, recovery from the neuromuscular block was complete within 30 seconds.

Variations in muscle temperature affected the neuromuscular blocking action of AH 10407. In the anaesthetized cat its potency and duration of action were greater in cooled muscle than in warmed muscle (Figure 2). In contrast, fazadinium and (+)-tubocurarine were more active and longer acting on the warmer muscle (Table 2).

Apnoea induced by AH 10407 in the cat was only seen with doses 3–4 times those required to inhibit tibialis anterior muscle twitches; for example, in one preparation 2 mg/kg caused 88% blockade of muscle twitches, but 6 mg/kg was required to arrest respiration. At this higher dose, muscle twitches were blocked for 87 s while respiration was arrested for only 12 seconds.

Table 1 Neuromuscular blocking potencies and time course of action of AH 10407 in the anaesthetized cat, dog, monkey and marmoset following intravenous injection

		Inhibition of tibialis muscle twitches (frequency, 1 Hz)				
Species (number of animals)	*Dose mg/kg (i.v.)*	*Time from injection to first detectable actions (s)*	*Time from detectable action to maximum effect (s)*	*Mean block (%)*	*Recover times (min:s) 50%*	*90%*
Cat (5)	0.5	6 (4–7)	3 (2–5)	14.3 (0–22.4)	0:04 (0–0:06)	0:06 (0–0:10)
	1.0	4.5 (3–6)	6 (5–7)	85.9 (83.3–88.4)	0:12 (0:08–0:15)	0:17 (0:12–0:25)
	2.0	5	5.5 (5–6)	100	0:28 (0:23–0:34)	0:35 (0:29–0:50)
Dog (2)	1.0	5 (4–6)	8 (5–9)	34.2 (25.2–40.0)	0:08 (0:02–0:16)	0:12 (0:05–0:20)
	2.0	5 (4–6)	7.5 (5–9)	73.8 (68.2–81.4)	0:14 (0:08–0:21)	0:17 (0:10–0:24)
	4.0	5 (4–6)	14 (9–15)	97.5 (85.0–100)	0:52 (0:45–0:59)	1:02 (0:49–1:12)
Cynamolgus monkey (2)	0.5	4 (3–5)	4 (3–5)	4.0 (0–8.0)	0:04 (0–0:06)	0:06 (0–0:10)
	1.0	4 (3–5)	7 (5–8)	67.0 (48.0–73.0)	0:15 (0:09–0:20)	0:19 (0:13–0:23)
	2.0	5 (3–7)	5 (4–6)	95.0 (89.2–100)	0:45 (0:35–0:50)	0:51 (0:38–1:00)
Cotton-eared marmoset (1)	1.0	5 (4–6)	14 (9–16)	50.9 (44.5–57.4)	0:26 (0:09–0:43)	0:47 (0:14–1:20)
	2.0	5 (4–6)	10 (8–13)	95.4 (94.4–96.4)	1:51 (1:02–2:20)	3:05 (2:59–3:12)

Values in the table refer to means (range) of responses.

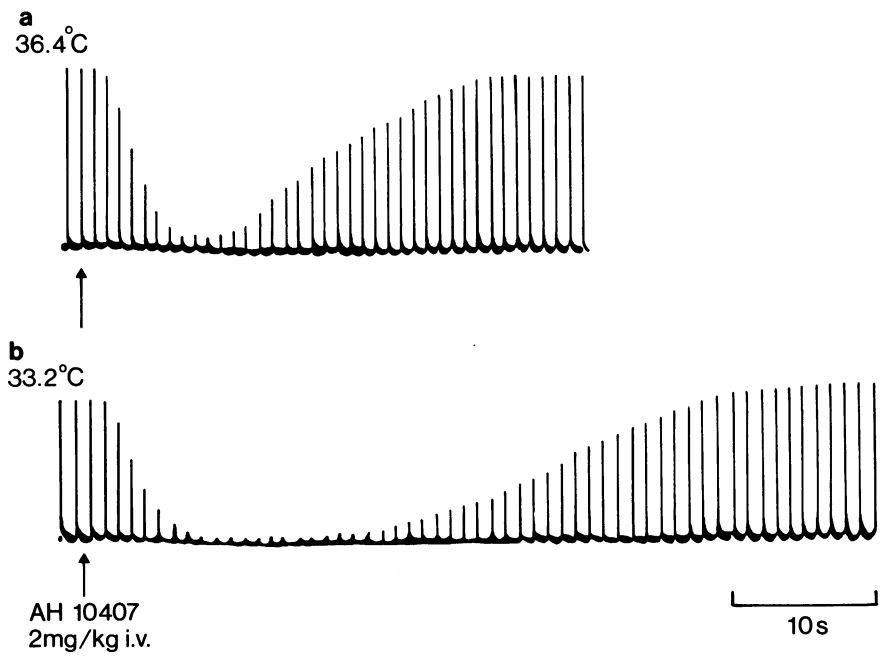


Figure 2 The effects of lowered muscle temperature on the neuromuscular blocking actions of AH 10407 in the cat anaesthetized with chloralose. The effects of an intravenous dose of AH 10407 on indirectly evoked twitches of the right tibialis anterior muscle (a) which was at 36.4°C and the left tibialis anterior muscle (b) which was cooled to 33.2°C, by packing the leg in ice, are compared in the same cat. Muscle temperature was measured with a deep muscle thermocouple probe.

Site and mechanism of action. The neuromuscular block induced by AH 10407 was primarily due to an action of the motor end-plate since doses of 0.5–4.0 mg/kg blocked twitches of the tibialis anterior muscle evoked by close-arterial injections of acetylcholine but not those evoked by direct electrical stimulation. The blockade was neither preceded by potentiation of the muscle twitch response nor associated with muscle fasciculations. Close-arterial injections of AH 10407, 100 μg, to the tibalis anterior muscle reduced indirectly evoked twitches and did not cause a contracture. The neuromuscular block induced by an intravenous infusion of AH 10407, 0.5 mg kg^{-1} min^{-1}, was rapidly and completely reversed by neostigmine. During partial block with AH 10407, muscle responses to tetanic stimulation

Table 2 The effects of muscle temperature on the neuromuscular blocking actions of fazadinium and (+)-tubocurarine in the anaesthetized cat

		Neuromuscular block	
Drug and dose (mg/kg i.v.)	*Muscle temp. (°C)*	*% Inhibition of tibialis muscle twitches*	*Duration of action (min)*
Fazadinium	31.5	41.3 ± 8.4	1.37 ± 0.33
(0.2 mg/kg)	36.0	64.9 ± 9.7	1.65 ± 0.53
	39.5	79.1 ± 10.7	2.10 ± 0.44
(+)-Tubocurarine	31.5	49.7 ± 11.1	10.2 ± 4.2
(0.2 mg/kg)	36.0	82.4 ± 12.7	12.4 ± 5.7
	39.5	100	16.7 ± 3.5

Results are means ± s.e. ($n = 4$)

were poorly sustained and there was a post-tetanic facilitation of the muscle twitches. No changes in the tibialis muscle surface potential occurred during AH 10407 blockade. The effects of competitive neuromuscular blocking drugs given concurrently with AH 10407 were additive, whereas depolarizing neuromuscular drugs antagonized the actions of AH 10407. All these characteristics show AH 10407 to be a competitive neuromuscular blocking drug.

Actions on the cardiovascular system in anaesthetized cats, dogs, Cynamolgus monkeys and marmosets

In artificially ventilated, anaesthetized cats neuromuscular blocking doses of AH 10407, 0.5–4.0 mg/kg intravenously, caused slight falls in mean arterial blood pressure (5–30 mmHg); 10 mg/kg caused a fall of 50 mmHg. These doses had no effect on the heart rate or ECG. The vasodepressor responses caused by AH 10407 lasted only 2–3 min but were slightly more persistent than the neuromuscular block. In artificially ventilated dogs, Cynamolgus monkeys and cotton-eared marmosets, single doses of AH 10407, 0.5–4 mg/kg intravenously, caused only minimal falls in blood pressure (5–15 mmHg). Prolonged intravenous infusions of 0.5 mg kg^{-1} min^{-1} in the anaesthetized cat reduced the blood pressure by 35 ± 14 mmHg and the heart rate by 25 ± 10 beats/minute.

In the anaesthetized cat, AH 10407, 0.5–2.0 mg/kg, caused dose-dependent inhibition (8–75%) of the hypotensive and negative chronotropic effects caused by periodic preganglionic stimulation of the vagus nerve. The inhibitory effect lasted for less than 60 seconds.

Effects on ganglionic transmission

In the anaesthetized cat the vasodepressor responses caused by AH 10407, 0.5–4.0 mg/kg intravenously, were accompanied by dose-dependent inhibition (20–50%) of contractions of the nictitating membrane evoked by periodic preganglionic sympathetic nerve stimulation; responses of the nictitating membrane to post-ganglionic stimulation of the cervical sympathetic nerve were unaffected.

In the dog anaesthetized with pentobarbitone, AH 10407, 2 and 10 mg/kg intravenously, inhibited vasopressor responses caused by DMPP 0.1 μg/kg intravenously, by 10 and 75% respectively. When these large doses of AH 10407 were repeated at intervals of less than 6 min a cumulative ganglion blocking action was seen which lasted for longer than the neuromuscular blockade.

Histamine release

In guinea-pigs anaesthetized with urethane AH 10407, 0.5–10 mg/kg intravenously, did not increase bronchial resistance whereas a substantial increase in bronchial resistance was seen after (+)-tubocurarine 0.2 mg/kg intravenously. Furthermore, in cats anaesthetized with chloralose, pretreatment with mepyramine 1 mg/kg, did not modify the vasodepressor responses induced by AH 10407. The results show that AH 10407 does not release histamine.

Neuromuscular blocking action on the isolated phrenic nerve-diaphragm preparation of the rat

AH 10407 inhibited diaphragm muscle twitches evoked by phrenic nerve stimulation. The EC_{50} (±s.e.) value for AH 10407 on this preparation was 9.5 ± 1.4 μg/ml. The course of investigation into the reasons for the termination of the neuromuscular blocking action of AH 10407 stemmed from the observation that the blocking effect of AH 10407 on the rat isolated phrenic nerve-diaphragm preparation terminated before the drug had been washed from the bathing fluid. Studies were therefore carried out to determine the chemical stability of AH 10407.

Chemical stability

AH 10407, dissolved in distilled water or bicarbonate-free Krebs solution, did not significantly decompose within 60 min at 37°C, but was unstable in Krebs solution and in dog and human whole blood, the half-life being about 1 to 2 minutes. The compound was somewhat more stable in Krebs solution containing sodium acetate which is less basic than sodium bicarbonate. These results are summarized in Figure 3. The rate of degradation of AH 10407 was, as expected, more rapid at higher than at lower incubation temperatures. For example, at temperatures of 24, 32 and 40°C AH 10407 was completely degraded in Krebs solution within 15, 5 and 1 min respectively.

It is clear from the results in Table 3 that AH 10407 was more stable in the more acidic solutions.

Analogues of AH 10407

The results given in the previous section show that AH 10407 is very susceptible to base-catalyzed degradation. A possible reaction mechanism, proposed by our colleagues, Dr J. Clitheroe and M. Wadsworth, is shown in Figure 4. The nucleophilic attack occurs at the electron deficient C_2 position and this results in opening of the imidazole ring and cleavage of the tetrazene chain (indicated by the broken lines a and b respectively). The proposed end-products are 3-methyl-2-phenyl-benzimidazole (2) and the azidobenzanilide (3).

If the mechanism in Figure 4 were correct, more stable compounds would be attained by reducing the electron deficit at C_2, perhaps by introducing electron-

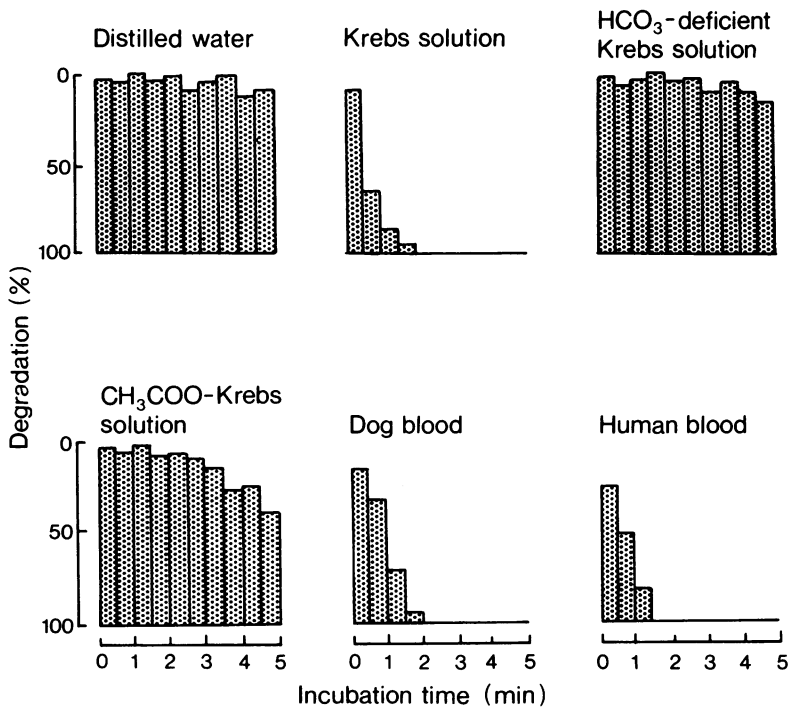


Figure 3 Rates of degradation of AH 10407 incubated *in vitro* at 37°C in various aqueous media. In Krebs physiological solution, dog blood and human blood AH 10407 was completely degraded within 2 minutes. In distilled water, Krebs solution deficient in HCO_3^- ions and in sodium acetate solution, AH 10407 was degraded much more slowly. The AH 10407 content in the incubates was assayed on the isolated phrenic nerve-diaphragm preparation of the rat.

donating groups at the corresponding *para*-position in the 2-phenyl substituent. Such compounds would be expected to be longer-acting than AH 10407 and, unlike that compound, might be stable enough to be used clinically; for the latter purpose they would also, of course, have to be selectively active at the motor end-plate.

As a result of this analysis, AH 11244 and AH 11056, the *p*-tolyl and *p*-methoxyphenyl analogues, were made and tested for stability in Krebs solution and neuromuscular blocking activity in the

Table 3 The effect of pH on the stability of AH 10407 in distilled water at 22°C

Incubation time (h)	*Degradation of AH 10407 (%)*		
	pH 6	*pH 3*	*pH 1*
1	0	0	0
3	0	0	0
5	0	0	0
7	26.9	0	0
24	75.7	8.0	0
48	100	10.0	0
168	—	100.0	0

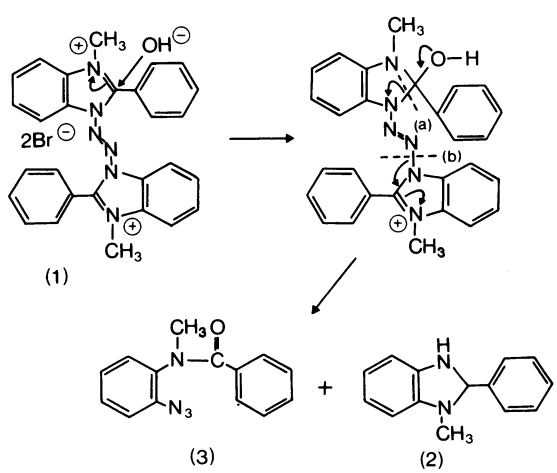


Figure 4 Proposed degradation pathway of AH 10407. The site of nucleophilic attack is the electron deficient C_2 position on one side of the molecule (1). The imidazole ring on that side opens (a) and there is a cleavage of the tetrazene chain (b) leaving the tertiary base (2) and the azidobenzanilide (3).

Table 4 Comparison of the rates of degradation of AH 10407, AH 11244 and AH 11056 incubated in normal Krebs solution at 37°C and their neuromuscular blocking potencies and durations of action in the anaesthetized cat

AH No.	Half-life in Krebs solution (min)	Inhibition of tibialis anterior muscle twitches (1 Hz) in the anaesthetized cat ED_{50} (mg/kg i.v.)	Duration of action (s)
10407	1.9	0.7	12
11244	2.3	0.8	16
11056	4.2	1.8	60

anaesthetized cat by methods already described. The results, summarized in Table 4, show that AH 11056 especially was more stable and longer-acting than AH 10407, results that are consistent with the starting hypothesis, since the methoxy group is a better electron donor than methyl. However, paralysing doses of AH 11056 were associated with marked falls in blood pressure in the anaesthetized cat, presumably due to ganglionic blockade, and so work on it was abandoned.

Discussion

1,1'-Azobis[3-methyl-2-phenylbenzimidazolinium]dimethanesulphonate, AH 10407, is a competitive neuromuscular blocking drug in the mouse, rat, cat, dog, Cynamologous monkey and cotton-eared marmoset. The drug has a rapid onset of action, but the most striking difference between this and other competitive neuromuscular blocking drugs is that its duration of action is extremely short. The most likely reason for this extremely short duration of neuromuscular block is that AH 10407 is chemically unstable in the presence of basic ions. Certainly the drug is rapidly degraded in physiological solutions and whole blood, yet it is relatively stable in distilled water and solutions which do not contain strongly basic ions. The rate of degradation of AH 10407 is also dependent upon the incubation temperature which may be the reason why the duration of neuromuscular block produced by AH 10407, unlike other competitive neuromuscular blocking drugs, is longer in cooled than in normothermic or warmed muscles. Furthermore, some analogues of AH 10407 which contain electron donating groups in the *para*-position of the phenyl rings are chemically more stable than AH 10407 and produce longer-lasting neuromuscular blockade in the anaesthetized cat.

Differences in the durations of action of AH 10407 in different species are more difficult to explain in terms of chemical instability. It is well known that competitive neuromuscular blocking drugs are longer-acting in primates than in lower animal species (Mushin & Mapleson, 1964; Hughes, 1972; Brittain & Tyers, 1972, 1973; Tyers, 1975). The same is also true for AH 10407 which is about five times longer-acting in the marmoset than in the cat and dog. A preliminary study in man (Blogg *et al.*, 1975) showed that the duration of action of AH 10407 would be similar to that found in the anaesthetized marmoset. It may be expected therefore that AH 10407 would be degraded at different rates in the different species. Surprisingly though, the degradation rates of AH 10407 in dog and human blood *in vitro* were very similar. It is clear therefore, that the rate of plasma clearance of AH 10407 cannot account for the termination of its neuromuscular blocking action. Furthermore, the very fast onset time to block for AH 10407 demonstrates that this drug can diffuse freely and rapidly across capillary barriers. It is unlikely therefore that the termination of action is limited by diffusional delays in clearing the drug from the extracellular fluid environment of the motor end-plate. It is postulated therefore that the rate of termination of action of AH 10407 is limited by the rate of dissociation of the drug from the acetylcholine receptors. However, the rate of chemical degradation also influences the duration of action of AH 10407 and its close analogues, AH 11056 and AH 11244, and thus it is tempting to propose that AH 10407 is degraded to inactive products whilst it is still bound to the acetylcholine receptors. The inactive degradation products would of course have to dissociate much more easily from the receptor than the parent molecule. The longer durations of action of AH 10407 in primates may then arise in two ways. First, the energy of activation for the dissociation of AH 10407 from the receptor is greater in primates than in lower species. This suggests that drug-receptor interactions differ qualitatively in different species and could involve exoreceptor sites in the binding of AH 10407. The increased binding energy may also reduce the susceptibility of bound AH 10407 to chemical degradation. An alternative, or perhaps complementary, explanation is that the amount of acetylcholine released from motor nerve endings is lower in primates than in the other species, since increased acetylcholine levels reduce or terminate the action of competitive neuromuscular blocking drugs on the motor end-plate. It is clear that to investigate these proposals it would be necessary to compare the reaction kinetics for the dissociation of AH 10407 from the receptor and for its degradation process.

On a broader basis, this hypothesis may also explain the species variations in the duration of action of other more stable, bis-quaternary competitive neuromuscular blocking drugs. For example, the rates of plasma clearance of the new neuromuscular blocking drug, fazadinium dibromide (AH 8165D) in the cat and man are very similar (Tyers, 1975), yet there is a con-

siderable difference in the duration of neuromuscular blockade induced by fazadinium in these species.

AH 10407 has clinically desirable properties but unfortunately, its inherent instability caused insuperable problems in its chemical development and pharmaceutical formulation. Nevertheless, base-catalyzed degradation is a potentially useful, novel, deactivating mechanism which may yet prove to be useful in the development of a competitive neuromuscular blocking drug that is genuinely short-acting in man.

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