Br. J. Pharmac. (1970), **38**, 503–516.

Studies on the blocking action of 2-(4-phenyl piperidino) cyclohexanol (AH5183)

I. G. MARSHALL

Department of Pharmacology, University of Strathclyde, Glasgow, C.1

Summary

1. AH5183 (2-(4-phenyl piperidino) cyclohexanol) produced neuromuscular block of slow onset in rapidly stimulated nerve-skeletal muscle preparations of the rat, chicken and cat.

2. The neuromuscular block was not antagonized by neostigmine, tetraethylammonium (TEA) or choline. The rate of onset of transmission failure was enhanced by factors which increase the release of acetylcholine.

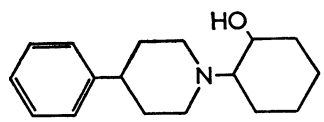
3. It was concluded that the neuromuscular blocking activity was primarily pre-junctional in origin, being due either to a non-competitive action on the choline transport mechanism, or to an intracellular action on acetylcholine metabolism.

4. In high doses AH5183 possessed local anaesthetic activity, but this was considered insufficient to bring about the failure of neuromuscular transmission.

5. AH5183 also produced a block of sympathetically innervated preparations that was indistinguishable from that produced by an α-adrenoceptor blocking drug.

Introduction

Potent neuromuscular blocking activity is not usually considered to be a property of tertiary bases such as 2-(4-phenyl piperidino) cyclohexanol (AH5183), yet Brittain, Levy & Tyers (1969a, b) found the compound to be active in this respect after both oral and parenteral administration in animals. Their results led them to conclude



AH5183

that AH5183 interrupts neuromuscular transmission through both a post-junctional tubocurarine-like action and a pre-junctional action through which transmitter output is reduced. The experiments described in this paper were undertaken to study the action of this compound in more detail.

Methods

The drug was tested on the following preparations.

(*a*) The isolated phrenic nerve-hemidiaphragm of the rat (Bülbring, 1946). In most experiments both hemidiaphragms from the same rat were mounted together

in Krebs-Henseleit solution at 32° C and of the following composition: (g/l.) NaCl 6·95, KCl 0·34, $CaCl_2$ 0·28, KH_2PO_4 0·162, $MgSO_4$ 0·294, $NaHCO_3$ 2·1, dextrose 2·0. The solution was continuously bubbled with 95% oxygen and 5% carbon dioxide. One muscle was excited once every second and the other once every 10 s, by rectangular pulses (100 μs duration) applied to the phrenic nerve. The strength of the shocks was about twice that required to evoke a maximal twitch.

(*b*) The isolated chick biventer cervicis muscle preparation (Ginsborg & Warriner, 1960). Maximal twitches of the isolated biventer cervicis muscle from chicks aged 4–8 days were elicited by stimulating the nerve within the muscle tendon with rectangular pulses of 100 μs duration and strength greater than that required to evoke a maximal twitch. Isometric contractions were recorded using a Statham G10B force transducer, connected to an ink-writing dynograph. The conditions of the experiments and the stimulation frequencies were identical with those used for the rat hemidiaphragm preparation.

(*c*) The tibialis anterior and soleus muscles of cats anaesthetized with a mixture of chloralose (80 mg/kg) and pentobarbitone sodium (2·5 mg/kg). Maximal twitches of ipsilateral soleus and tibialis anterior muscles were elicited once every second by stimulation of the sciatic nerve with rectangular pulses of 100 μs duration. Isometric contractions were recorded by Grass FT03C and FT10C force tranducers connected to an ink-writing dynograph. Arterial blood pressure was recorded in mm Hg (1 mm Hg$\equiv$1·333 mbar) from a common carotid artery by a Statham pressure transducer, and in some experiments respiration was measured by a thermistor probe in the trachea.

(*d*) Local anaesthetic activity. (i) AH5183 was compared with lignocaine using the guinea-pig weal test of Bülbring & Wajda (1945). (ii) The action of AH5183 on gross nerve action potentials recorded from the isolated rat phrenic nerve was compared with those of lignocaine and procaine. Rats were killed by a blow on the head and 5–6 cm of the left phrenic nerve was dissected free and set up in a Perspex triple chamber similar to that described by Crankshaw & Raper (1968). Each end of the nerve was immersed in liquid paraffin previously equilibrated with Krebs-Henseleit solution and bubbled with oxygen containing 5% carbon dioxide. The central portion of the nerve was immersed in Krebs-Henseleit solution bubbled with oxygen and carbon dioxide in a chamber isolated from the outer chambers by seals of silicone high vacuum grease. The two ends of the nerve were in contact with stimulating electrodes and platinum recording electrodes respectively, while the fluid of the central chamber was in contact with an indifferent earthing electrode. The nerve was stimulated at a frequency of 5 Hz with rectangular pulses of 100 μs duration and of strength greater than that required to produce a maximal action potential. Action potentials were amplified and displayed on an oscilloscope.

(*e*) The isolated intestine of the rabbit (Finkleman, 1930). Spontaneous pendular movements were recorded from isolated segments of ileum mounted in Krebs solution at 37° C and of the following composition: (g/l.) NaCl 6·95, KCl 0·35, $CaCl_2$ 0·28, NaH_2PO_4 0·154, $MgCl_2$ 0·115, $NaHCO_3$ 2·1, dextrose 2·0. The solution was continuously bubbled with 95% oxygen and 5% carbon dioxide. Inhibition of the pendular movements was produced by stimulation of the periarterial nerve supply every six minutes by rectangular pulses (0·5 ms duration) at a frequency of 5–20 Hz for 30 s. Between each period of nerve stimulation inhibition of

pendular movements was produced by the addition of a sympathomimetic amine to the bathing fluid, the period of contact being 60 s. The segments of intestine were suspended under a resting load of 3 g, so that little tone was present in the tissue. Pendular movements were recorded isotonically on a smoked surface.

(*f*) The isolated rabbit ear artery (De la Lande & Rand, 1965). The central ear artery was dissected free of surrounding tissues and cannulated with a fine polythene cannula. A length of 3–5 cm of artery was excised and perfused with McEwen's (1956) solution gassed with 95% oxygen and 5% carbon dioxide at 37° C. Perfusion pressure was measured with a Condon mercury manometer and recorded on smoked paper. The periarterial nerves were stimulated by rectangular pulses (1 ms duration) at rates of 5–10 Hz for a period of 10 s every 3 min. Responses to noradrenaline were obtained by injecting the drug dissolved in 0·1 ml of McEwen's solution into the perfusion fluid just before it reached the cannula. Antagonist drugs were added directly to the reservoir.

Drugs used were: acetylcholine chloride, carbachol chloride, choline chloride, chloralose, quinidine, tetraethylammonium bromide (British Drug Houses), (+)-tubocurarine chloride (Burroughs Wellcome), hemicholinium No. 3 (Aldrich Chemical), triethylcholine iodide (Ward Blenkinsop), neostigmine methylsulphate (Roche), lignocaine hydrochloride (Duncan Flockhart), pentobarbitone sodium (Abbott), (−)-noradrenaline, (−)-phenylephrine, sodium adenosine-5′-triphosphate (ATP-Sigma), guanethidine methylsulphate, phentolamine (Ciba), procaine hydrochloride (May & Baker), (−)-isoprenaline bitartrate (Wyeth). The doses quoted refer to the salts or the bases.

Results

Rat phrenic nerve-hemidiaphragm preparations

(a) Effect of stimulation frequency

Low doses of AH5183 (2–6 μg/ml) were either without effect or produced a small (up to 20%) augmentation of twitch tension when the frequency of stimulation was 0·1 Hz (Figs. 1, 2 and 3). In fresh preparations stimulated at 1 Hz, the small augmentation of twitch tension was followed by a slowly developing block which reached 59·0 ± 3·9% (mean ± S.E.M.) reduction of twitch height in 30 min. The time between addition of the drug (5 μg/ml) and onset of block in six experiments was 10–15 min (mean ± S.E.M. was 13·7 ± 1·9) (Figs. 1, 2 and 3). After washing the AH5183 from the bath, subsequent additions of smaller amounts (2–3 μg/ml) of the same drug produced an equivalent block of much more rapid onset (Fig. 4). Slowing the stimulation rate to 0·1 Hz during the block of transmission produced an immediate but transient reversal of the block which gradually redeveloped even at the lower stimulation rate (Fig. 1).

In order to block preparations continually stimulated at a frequency of 0·1 Hz doses 10–12 times greater than those that blocked the preparations stimulated at a frequency of 1 Hz were required. The dependence of the degree of blockade on the rate of stimulation was also evident in an experiment in which one hemidiaphragm was stimulated at 1 Hz while the contralateral hemidiaphragm was stimulated at 0·5 Hz. AH5183 (2 μg/ml) produced a 93% reduction of the twitches of the former preparation, but only a 25% reduction in the latter preparation. On washing preparations stimulated continuously at a frequency of 1 Hz and blocked

by AH5183, no recovery of twitch height was observed until the stimulation rate was reduced to 0·1 Hz, after which full recovery occurred within 15–25 min.

The effects of tetanic stimulation of the phrenic nerve (50 Hz for 10 s) were studied during transmission failure produced by AH5183 and compared with the effects during block by tubocurarine and the pre-junctionally active drugs hemicholinium-3 (HC-3) and triethylcholine (TEC). During the block of the more rapidly stimulated of the two hemidiaphragm preparations produced by AH5183 (2–5 μg/ml), HC-3 (35 μg/ml) or TEC (300–400 μg/ml), the tension of a tetanus in the rapidly stimulated muscle was reduced and fell throughout the period of stimulation. Post-tetanic augmentation of twitch tension was generally present for 5–10 s in the preparations blocked by AH5183, HC-3 and TEC. In the contralateral muscle, stimulated at 0·1 Hz, the tension of the tetanus was somewhat reduced but it was maintained during the period of stimulation (Fig. 1). In contrast, during the block of a rapidly stimulated hemidiaphragm preparation produced by tubocurarine (0·5

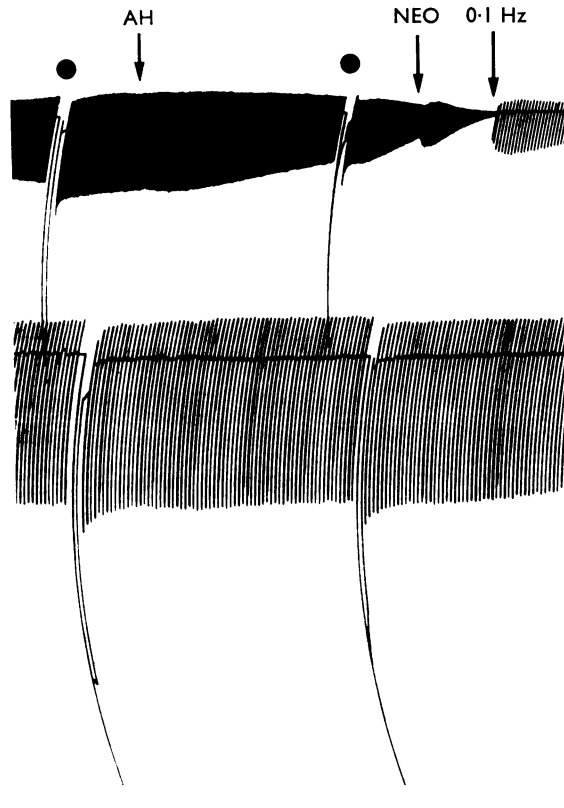


FIG. 1. Rat phrenic nerve-hemidiaphragm preparations. Both hemidiaphragms from the same rat were mounted in the same organ bath and maximal twitches were elicited indirectly. Contractions and tetani are downwards. The upper preparation was stimulated at 1 Hz and the lower at 0·1 Hz. At the solid circles, tetanic stimulation (50 Hz for 10 s) was delivered via the phrenic nerve. At AH, AH5183 (5 μg/ml) and at NEO, neostigmine (1 μg/ml) were added to the bath. At 0·1 Hz, the rate of stimulation of the upper preparation was slowed from 1 Hz to 0·1 Hz. The horizontal bar corresponds to 5 min. The neuromuscular blocking action of AH5183 was dependent on the frequency of nerve stimulation, tetanic tension was poorly maintained in the blocked preparation, and neostigmine exhibited only transient antagonistic activity.

μg/ml), the tension of a tetanus was reduced to an equal degree in both the blocked muscle and the more slowly stimulated, apparently unaffected, contralateral muscle, and the tensions of the tetani were reduced similarly in both muscles. Post-tetanic decurarization was not pronounced in this isolated preparation, and lasted for only a period of 5–20 seconds.

Because of the initial augmentation of maximal twitches often produced by AH5183, it was tested for anti-curare action. In concentrations up to 12 μg/ml, however, it was completely without effect on twitches evoked at a frequency of 0·1 Hz and partially blocked by tubocurarine (0·5 μg/ml).

(b) Effects of antagonists

(1) *Choline.* Choline (10–60 μg/ml), added during block of the rapidly stimulated diaphragms produced by AH5183 (2–6 μg/ml), accelerated the rate of onset and degree of transmission failure (Fig. 2). Even after washing the AH5183 from the bath, choline (60 μg/ml) caused an increase in the degree of the residual block. When added to the bath 10 min before the addition of AH 5183, choline (10–200 μg/ml) augmented the blocking action of a standard dose of AH5183 (2–3 μg/ml). Washing a tissue blocked by AH5183 and then treated with choline, however, occasionally produced a more rapid recovery of twitch tension than did washing the same tissue which had been treated with AH5183 alone. Choline (60 μg/ml) reversed the blocking action of TEC (250–400 μg/ml), and prior administration of choline (100 μg/ml) diminished the blocking action of HC-3 (40 μg/ml) as demonstrated also by other workers (Bowman & Rand, 1961 ; Bowman, Hemsworth & Rand, 1967).

(2) *Neostigmine.* During transmission failure in both rapidly and slowly stimulated hemidiaphragms produced by small and large doses of AH5183 respectively,

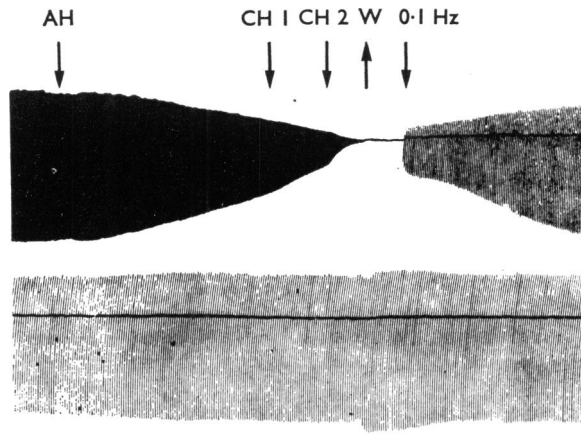


FIG. 2. Rat phrenic nerve-hemidiaphragm preparations as in Fig. 1. At AH5183 (5 μg/ml), at CH 1, choline (10 μg/ml), and at CH 2, choline (50 μg/ml), were added to the bath. At W the preparation was washed for 60 s with fresh Krebs-Henseleit solution, and at 0·1 Hz the rate of stimulation of the upper preparation was slowed from 1 Hz to 0·1 Hz. The horizontal bar corresponds to 10 min. The blocking action of AH5183 was enhanced by choline and recovery was dependent on slowing the stimulation rate after washout.

neostigmine (0·5–1 μg/ml) produced only a weak and transient antagonism of the block (Fig. 1). Similar effects of neostigmine were observed during block of rapidly stimulated hemidiaphragms produced by HC-3 (40 μg/ml). In contrast, neostigmine (0·5 μg/ml) completely and permanently reversed neuromuscular block produced by tubocurarine (0·5 μg/ml) in both slowly and rapidly stimulated hemidiaphragms.

(3) *Tetraethylammonium.* The main action of tetraethylammonium (TEA) at the neuromuscular junction is to increase the release of acetylcholine from the nerve terminals (Stovner, 1957, 1958 ; Koketsu, 1958 ; Collier & Exley, 1963). This action accounts for its ability to reverse neuromuscular block produced by tubocurarine or by low Ca^{2+} levels. When added to a rapidly stimulated hemidiaphragm during block produced by AH5183, TEA (200 μg/ml) produced an immediate but transient relief of the block. However, this was followed by a rapid increase in the rate of depression of twitches.

(c) Changes in ionic concentrations

In these experiments the hemidiaphragm preparations were exposed to the changed solutions for 10 min before the addition of AH5183. During the first 5 min of this period the preparation was stimulated at a frequency of 0·1 Hz. The frequency was then increased to 1 Hz, 5 min before the addition of AH5183.

The results, which are summarized in Table 1, showed that elevated calcium and depressed magnesium levels significantly shortened the latent period and accelerated the rate of development of the block due to AH5183. Elevated magnesium and

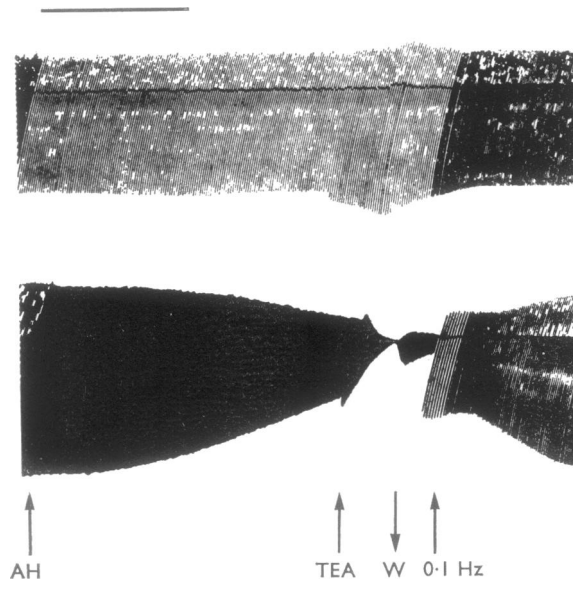


FIG. 3. Rat phrenic nerve-hemidiaphragm preparations as in Fig. 1. At AH, AH5183 (5 μg/ml) and at TEA, tetraethylammonium (200 μg/ml) were added to the bath. At W, the preparation was washed with fresh Krebs-Henseleit solution, and at 0·1 Hz, the rate of stimulation was slowed from 1 Hz to 0·1 Hz (90 s after 0·1 Hz, the kymograph drum speed was halved). The horizontal bar corresponds to 10 min. TEA exhibited a transient antagonistic action, after which it rapidly increased the rate of development of the block.

depressed calcium levels prolonged the latent period and slowed the rate of development of block although in this case the changes in latent period were not statistically significant.

(d) Comparison with local anaesthetic agents

The effects described in the previous sections suggested that AH5183 had a prejunctional blocking action and for this reason it was compared with the membrane stabilizing drugs, lignocaine, procaine and quinidine. Lignocaine (65–70 μg/ml) and procaine (100 μg/ml) produced a neuromuscular block in the rat hemidiaphragm preparation similar, in most respects, to that produced by tubocurarine. For example, although both the slowly and rapidly stimulated preparations were affected, the block in the rapidly stimulated muscle was more pronounced, and a tetanus (50/Hz for 10 s) was poorly maintained in both muscles. Post tetanic twitch augmentation was transient and similar to that noted after addition of the hemicholinium-like compounds. Rapid recovery of twitch tension occurred when the local anaesthetics were washed from the bath. Unlike that produced by tubocurarine, however, the block of transmission produced by lignocaine was completely unaffected by the addition of neostigmine (1 μg/ml). Quinidine (20–40 μg/ml) augmented the twitch tension in both the rapidly and slowly stimulated preparations, presumably due to its action in prolonging the active state of the stimulated muscle (Lammers & Ritchie, 1955). However, quinidine 40 μg/ml produced a secondary diminution of twitch tension in the more rapidly stimulated preparation, during which time tetani were poorly maintained in both muscles.

TABLE 1. *Effects of alteration of external ionic environment on the blocking action of AH*5183 (5 μg/ml) *in the rat hemidiaphragm stimulated at* 1 *Hz*

Ionic environment	Latent period (min±S.E.M.)	*P*	% block of twitch height in 30 min (±S.E.M.)	*P*
AH 5183+normal Krebs-Henseleit	13·7±2·0		59·0±3·9	
AH 5183+2×Ca^{2+} Krebs-Henseleit	6·7±1·8	>0·001	95·8±7·3	>0·001
AH 5183+0·25×Ca^{2+} Krebs-Henseleit	15·3±2·5	>0·01	43·3±7·2	>0·001
AH 5183+2×Mg^{2+} Krebs-Henseleit	14·3±4·7	<0·25	49·3±4·9	>0·001
AH 5183+0·25×Mg^{2+} Krebs-Henseleit	10·0±3·1	>0·001	68·1±3·8	>0·001

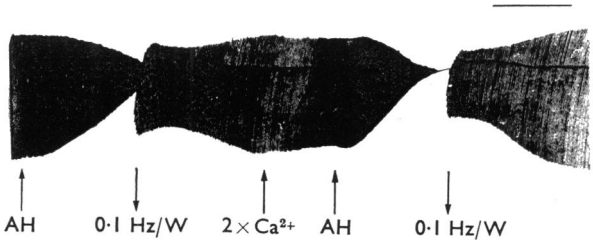


FIG. 4. Rat hemidiaphragm preparation stimulated at 1 Hz. At AH, AH5183 (3 μg/ml) was added to the bath. At 2×Ca^{2+}, the Krebs-Henseleit solution was replaced by Krebs-Henseleit solution containing twice the normal amount of calcium chloride. At 0·1 Hz/W the stimulation rate was slowed from 1 Hz to 0·1 Hz and the preparation was simultaneously washed with normal Krebs-Henseleit solution. In each case the time interval between AH and 0·1 Hz was exactly 15 min. The horizontal bar corresponds to 10 min. The blocking action of AH5183 was enhanced in the presence of elevated levels of calcium ions. Previous additions of AH5183 to the preparation had illustrated that successive equal doses produced approximately the same degree of block when tested under the above conditions.

Chick biventer cervicis preparations

Essentially similar results to those obtained with the rat hemidiaphragm were observed in experiments on the chick biventer cervicis muscle preparation. AH5183 was slightly less potent in the chick preparation than in the rat hemidiaphragm, but otherwise the characteristics of the block were similar. Variation of the external Ca^{2+} and Mg^{2+} concentrations also produced similar effects to those noted in the rat hemidiaphragm. The actions of the anti-curare substances, neostigmine (0·5–1 μg/ml) and TEA (200 μg/ml) were more pronounced than in the rat hemidiaphragm. Both of these compounds usually produced a complete, although transient, reversal of the block produced by AH5183 in the chick muscle. The block then redeveloped after about 60 seconds. Choline (30–100 μg/ml) was without antagonistic activity. Recovery of twitch height after washing out AH5183 was extremely slow (1–2 h) in this preparation even at a stimulation rate of 0·1 Hz.

Effects on responses to acetylcholine and carbachol

In these experiments the nerve was stimulated at a frequency of 1 Hz. Periodically throughout the experiment, electrical stimulation was temporarily stopped and either acetylcholine or carbachol was added to the bath in a concentration sufficient to produce a contracture approximately equal in amplitude to that of the twitches. Twenty minutes after the addition of the blocking drug, the percentage block of twitches and of acetylcholine or carbachol responses was measured. The ratios of the percentage block of twitches (T): percentage block of acetylcholine response (ACh), and of the percentage block of twitches (T): the percentage block of carbachol response (Carb), produced by the first addition of AH5183 were calculated. A ratio greater than 1 means that the twitch was depressed to the greater extent and vice versa. It was previously found in this tissue that post-junctionally active drugs such as tubocurarine block responses to carbachol more effectively than responses to acetylcholine (Marshall, 1969) and for this reason carbachol was used in most experiments.

The values obtained with AH5183 were T : ACh=5·5–20 (mean 13·0, three experiments) and T : Carb=2·25–13·0 (mean 6·0, six experiments). The large ranges occurred because, in a few experiments, AH5183 produced less than 5% block of the response to acetylcholine or carbachol in concentrations that produced an almost complete block of twitch height. The corresponding T : Carb figure for lignocaine ranged from 1·0–4·1 (mean 1·85, seven experiments).

Cat nerve-muscle preparations

When injected intravenously into cats anaesthetized with chloralose, AH5183 (0·75–1·0 mg/kg) produced an immediate but transient drop in blood pressure and occasionally a cessation of respiration. After these initial effects the blood pressure remained 10–20 mm Hg below normal and respiration was shallow for approximately 60 min.

No immediate effect of the drug was noted either on the rapidly stimulated soleus or tibialis anterior muscle. Any tubocurarine-like action of the compound would be expected to be exhibited immediately, as the soleus muscle in particular (Paton

& Zaimis, 1951) and rapidly stimulated muscles generally (Preston & van Maanen, 1953 ; Wislicki, 1958) are especially sensitive to blocking drugs of this type.

After a latent period of 2–5 min AH5183 produced a slowly developing block of the contractions of both the soleus and tibialis anterior muscles, the maximum degree of block being produced after 20–25 min (Fig. 5). Recovery from the block proceeded slowly, full recovery occurring after 40–60 min. In common with the hemicholinium-like class of drugs (Bowman & Rand, 1961), AH5183 always produced a greater degree of block in the tibialis anterior muscle than in the ipsilateral soleus muscle. When choline 5 mg/kg was injected intravenously at the height of the block an immediate but transient reversal of the block was seen (Fig. 5), the twitch height returned to its previous depressed level within 2–3 min. Recovery from the blocking action of AH5183 did not appear to proceed any more rapidly after the injection of choline.

Local anaesthetic activity

Although the experiments suggested that AH5183 possessed a mode of action different from that of lignocaine, it was considered possible that it could be exerting its pre-junctional blocking action by a local anaesthetic action. Accordingly the relative local anaesthetic potencies of AH5183 and lignocaine were calculated and compared with the relative potencies of the two compounds in blocking neuromuscular transmission.

Initial testing of AH5183 using the guinea-pig weal method indicated that AH5183 possessed local anaesthetic activity of a lesser degree or more transient duration than that produced by lignocaine. More quantitative estimates of the compounds' local anaesthetic activities were obtained by comparing their action

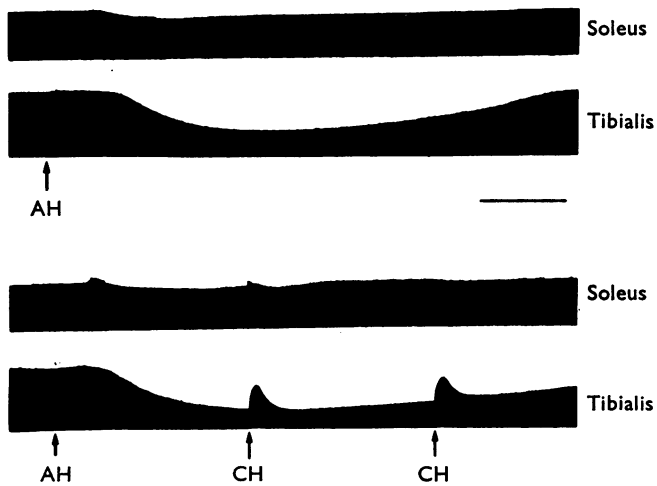


FIG. 5. Cat tibialis anterior (lower) and soleus (upper) muscle preparations from the same leg stimulated at 1 Hz via the sciatic nerve. At AH, AH5183 (1 mg/kg), and at CH in the lower record, choline (5 mg/kg) were injected intravenously. The lower traces were recorded 60 min after the upper. After a latent period of 5 min AH 5183 produced a neuromuscular block of slow onset in the tibialis anterior muscle, and a much shallower and shorter lasting block in the soleus muscle. Choline produced a transient antagonism of the block of the tibialis anterior muscle, but has no effect on the rate of recovery from the blocking action of AH5183. The horizontal bar corresponds to 10 min.

on gross nerve action potentials recorded from the rat phrenic nerve *in vitro*. The amplitudes of the action potentials were recorded before and 10 min after the addition of the drugs to the fluid bathing the central portion of the nerve.

Lignocaine in concentrations of 65–75 μg/ml, procaine in concentrations of 100–120 μg/ml and AH5183 in concentrations of 60–75 μg/ml were approximately equiactive in reducing the amplitude of the action potentials. These same concentrations of lignocaine and procaine were those found effective in depressing the amplitude of twitches of the rat diaphragm. However, these concentrations of AH5183 were 10–20 times greater than those required to reduce twitch height in the hemidiaphragm. Low doses of AH5183 (5–20 μg/ml) that blocked the twitches were without obvious effect on the size of the action potentials.

Sympathetically innervated preparations

In both the intestine and the ear artery of the rabbit AH5183 (2–5 μg/ml) reduced the sizes of the responses to stimulation of the periarterial nerve supply. In contrast to its action on cholinergic preparations, the onset of action was immediate and rapid recovery occurred on washing. The degree of block was independent of the frequency of stimulation. In the ear artery the responses to noradrenaline (50 ng) and to nerve stimulation were reduced to a similar extent. Control experiments showed that at concentrations which produced a 75% inhibition of the responses to electrical stimulation, phentolamine (1 μg/ml) reduced noradrenaline responses to approximately the same extent, and guanethidine (0·5 μg/ml) augmented them.

ATP produces inhibition of pendular movements in rabbit intestine, the effect superficially resembling that of a catecholamine (Drury, 1936). Equivalent inhibitory responses were produced by phenylephrine (0·1 μg/ml), isoprenaline (0·02 μg/ml) and ATP (1·2 μg/ml), and by periarterial nerve stimulation. AH5183 (5 μg/ml) reduced the responses to nerve stimulation. At this concentration the response to phenylephrine was reduced to a greater degree than that to nerve

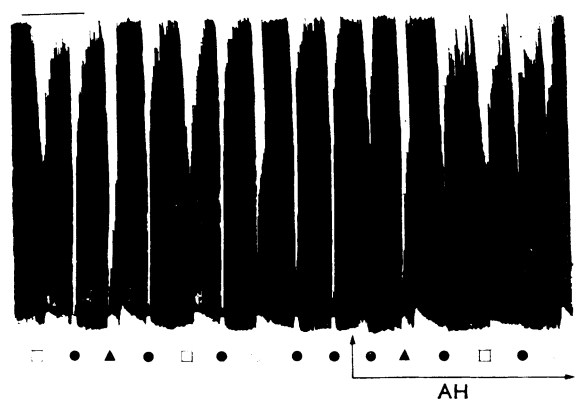


FIG. 6. Isolated rabbit intestine. At the solid circles, the preparation was stimulated via the periarterial nerve supply (10 Hz for 30 s). At the triangles ATP (1·2 μg/ml), at the squares isoprenaline (0·02 μg/ml) and at the open circles phenylephrine (0·1 μg/ml) were added to the bath for a period of 60 s. During the period marked by the arrows AH5183 (5 μg/ml) was present in the bathing fluid. The horizontal bar corresponds to 5 min. In the presence of AH5183 the responses to nerve stimulation and to phenylephrine were depressed, whereas those to isoprenaline and ATP were unaffected.

stimulation, while the responses to isoprenaline and ATP were unaffected (Fig. 6). Similar effects were produced by phentolamine (1 μg/ml). Quinidine differed in its effects from both AH5183 and phentolamine. In concentrations of 20 μg/ml quinidine blocked responses both to phenylephrine and to ATP, reduced those to nerve stimulation, and left those to isoprenaline unchanged.

Discussion

The results show that the actions of AH5183 are not confined to cholinergic neuroeffector transmission sites. In addition the compound blocked adrenergic transmission by an action that was indistinguishable from that of an α-adrenoceptor blocking drug, and it also possesses local anaesthetic activity. Local anaesthetic drugs, including quinidine, have been shown to block responses to α-adrenoceptor agonists, but they may be distinguished from the more specific α-adrenoceptor blocking drugs by the fact that they also block responses to ATP, whereas the latter drugs do not (Bowman & Hall, 1970). Although AH5183 possesses local anaesthetic activity, this effect did not appear to account for its ability to block adrenergic transmission or responses to phenylephrine, because the effective concentrations were considerably lower than those necessary to block the conduction of action potentials in the isolated phrenic nerve, and concentrations effective in blocking responses to phenylephrine were without effect on responses to ATP.

For similar reasons, the neuromuscular blocking activity of AH5183 did not appear to be a consequence of its local anaesthetic activity. Again the effective neuromuscular blocking doses were much lower than those necessary to block nerve conduction, whereas with the true local anaesthetics (lignocaine, procaine) the effective concentrations for the two effects were the same. Furthermore, the characteristics of the block produced by AH5183 were quite different from those of a block produced by the local anaesthetic drugs, including quinidine.

The block of adrenergic transmission differed in its time course from that of cholinergic transmission, and, unlike the latter, was independent of stimulation frequency, indicating that the two effects are not a consequence of the same basic mechanism of action. It is therefore concluded that AH5183 has at least three independent actions—a blocking action at cholinergic junctions, an α-adrenoceptor blocking action and, in higher concentrations, a local anaesthetic action.

The evidence suggested that in skeletal muscle, AH5183 interrupts transmission mainly by a pre-junctional mechanism. Thus, in the chick biventer cervicis muscle it was considerably more effective in blocking responses to carbachol than in blocking neurally evoked twitches. This test provides a sensitive method for detecting post-junctional blocking activity (Marshall, 1969). AH5183 in concentrations that depressed the twitches was less effective in blocking carbachol contractures than were any of the drugs studied under identical conditions in a previous series of experiments (Marshall, 1969), even though the drugs used previously included hemicholinium-3 and troxypyrrolium. Troxypyrrolium was the most selective of the drugs previously shown to block transmission by a pre-junctional mechanism in this preparation. Additional observations indicative of a pre-junctional site of action of AH5183 were the striking dependence of the degree of block on the frequency of stimulation, the weak antagonistic action of the anticurare drugs, TEA and neostigmine, and the fact that in the cat, the soleus muscle was more resistant than the tibialis anterior. These characteristics are also observed

with the hemicholinium series of drugs (see Schueler, 1960 ; Bowman *et al.*, 1967, for reviews). Furthermore, Ca^{2+} and Mg^{2+}, which are additive in their effects on the post-junctional membrane (Takeuchi, 1963), are antagonistic in their effects on transmitter release (del Castillo & Engbaeck, 1954). These ions influenced block by AH5183 in opposite ways, thus again pointing to a pre-junctional site of action of this compound. From these results it therefore appears that, in the skeletal muscles studied, AH5183 has little post-junctional blocking activity. This conclusion differs from that of Brittain *et al.* (1969a, b), who considered a component of post-junctional action of the tubocurarine type to play a significant role in the action of AH5183.

Procedures or drugs known to increase the release of transmitter, for example elevated Ca^{2+}, lowered Mg^{2+} (Hubbard, Jones & Landau, 1968), or TEA (Collier & Exley, 1963), enhanced rather than antagonized the rate of development of neuromuscular blockade produced by AH5183, indicating that the drug does not directly inhibit the release mechanism. The lack of anti-tubocurarine or twitch-augmenting activity in the chick biventer cervicis preparation, which is extremely sensitive to drugs that increase the release of acetylcholine (Marshall, 1969), indicates that AH5183 does not block transmission by depleting the terminal stores of acetylcholine through excessive release. Indeed the presence of a latent period suggests that it is necessary to deplete the terminal stores by rapid nerve stimulation before the onset of transmission failure may be observed.

In the rat and chick experiments no evidence of antagonism by choline was observed. In the cat tibialis anterior muscle choline produced only a transient reversal of AH5183-induced transmission failure, which resembled that observed during block by tubocurarine. The effect bore little resemblance to the striking and permanent antagonism produced by choline against drugs that block the choline transport mechanism (Bowman *et al.*, 1967). It therefore appears that AH5183 either does not act on the choline transport mechanism, or that any action at this site is non-competitive in nature. The weak antagonistic action of choline against AH5183 may be ascribed either to its depolarizing action or to its ability to increase transmitter release without itself being incorporated into acetylcholine (Hutter, 1952 ; Blaber & Bowman, 1959).

The delayed onset of action, slow recovery after washing the tissue, and the non-quaternary nature of AH5183 suggest that the compound may be able to penetrate biological membranes to exert an intracellular action on acetylcholine synthesis or storage. If this is so, it apparently does not act by direct inhibition of choline acetyltransferase (Brittain *et al.*, 1969b). A reduction in the supply of acetyl co-enzyme A or ATP for acetylcholine synthesis remains a possibility, but appears unlikely in view of the necessity for these substances in other biochemical reactions throughout the body, which are apparently unaffected by AH5183. Choline acetylation is now believed to occur in the soluble cytoplasm of the nerve endings (Fonnum, 1967), after which the quaternary acetylcholine presumably passes through the synaptic vesicle membrane probably by some transport mechanism. It is not unreasonable to suppose that this mechanism may be susceptible to drug action, as is the choline transport mechanism. Thus by probable elimination of other possible pre-junctional sites of action and taking into account its chemical structure and time course of action it appears that AH5183 may inhibit the uptake of newly synthesized acetylcholine into the storage vesicles, thereby producing an

eventual failure of transmission. AH5183 may therefore prove to be a useful tool for further pharmacological dissection of the pre-junctional events in neuromuscular transmission.

I thank Dr. R. T. Brittain (Allen & Hanburys) for the gift of AH5183 and for access to his manuscript, and Professor W. C. Bowman for helpful discussion of the manuscript. The skilled technical assistance of Mrs. P. Wilson and Miss R. E. Jolley is gratefully acknowledged. Part of this work was supported by a grant from the British Egg Marketing Board.

REFERENCES

BLABER, L. C. & BOWMAN, W. C. (1959). A comparison between the effects of edrophonium and choline in the skeletal muscles of the cat. *Br. J. Pharmac. Chemother.*, **14**, 456–466.

BOWMAN, W. C. & HALL, M. T. (1970). Inhibition of rabbit intestine mediated by α- and β-adrenoceptors. *Br. J. Pharmac.*, **38**, 399–415.

BOWMAN, W. C., HEMSWORTH, B. A. & RAND, M. J. (1967). Effects of analogues of choline on neuromuscular transmission. *Ann. N.Y.. Acad. Sci.*, **144**, 471–481.

BOWMAN, W. C. & RAND, M. J. (1961). Actions of triethylcholine on neuromuscular transmission. *Br. J. Pharmac. Chemother.*, **17**, 176–195.

BRITTAIN, R. T., LEVY, G. P. & TYERS, M. B. (1969a). Observations on the neuromuscular blocking action of 2-(4-phenylpiperidino)-cyclohexanol. *Br. J. Pharmac.*, **36**, 173–174*P*.

BRITTAIN, R. T., LEVY, G. P. & TYERS, M. B. (1969b). The neuromuscular blocking action of 2-(4-phenylpiperidino)-cyclohexanol (AH5183). *Eur. J. Pharmac.*, **8**, 93–99.

BÜLBRING, E. (1946). Observations on the isolated phrenic-nerve diaphragm preparation of the rat. *Br. J. Pharmac. Chemother.*, **1**, 38–61.

BÜLBRING, E. & WAJDA, I. (1945). Biological comparison of local anaesthetics. *J. Pharmac. exp. Ther.*, **85**, 78–84.

COLLIER, B. & EXLEY, K. A. (1963). Mechanism of the antagonism by tetraethylammonium of neuromuscular block due to *d*-tubocurarine, or calcium deficiency. *Nature, Lond.*, **199**, 702–703.

CRANKSHAW, D. P. & RAPER, C. (1968). Some studies on peripheral actions of mephenesin, methocarbamol and diazepam. *Br. J. Pharmac.*, **34**, 579–590.

DE LA LANDE, I. S. & RAND, M. J. (1965). A simple isolated nerve-blood vessel preparation. *Aust. J. exp. Biol. med. Sci.*, **43**, 639–656.

DEL CASTILLO, J. & ENGBAECK, L. (1954). The nature of the neuromuscular block produced by magnesium. *J. Physiol. Lond.*, **124**, 370–384.

DRURY, A. N. (1936). The physiological activity of nucleic acid and its derivatives. *Physiol. Rev.*, **16**, 292–325.

FINKLEMAN, B. (1930). On the nature of inhibition in the intestine. *J. Physiol., Lond.*, **70**, 145–157.

FONNUM, F. (1967). The "compartmentation" of choline acetyltransferase within the synaptosome. *Biochem. J.*, **103**, 262–270.

GINSBORG, B. L. & WARRINER, J. (1960). The isolated chick biventer cervicis nerve-muscle preparation. *Br. J. Pharmac. Chemother.*, **15**, 410–411.

HUBBARD, J. I., JONES, S. F. & LANDAU, E. M. (1968). On the mechanism by which calcium and magnesium affect the release of transmitter by nerve impulses. *J. Physiol., Lond.*, **196**, 75–86.

HUTTER, O. F. (1952). Effect of choline on neuromuscular transmission in the cat. *J. Physiol., Lond.*, **117**, 241–250.

KOKETSU, K. (1958). Action of tetraethylammonium chloride on neuromuscular transmission in the frog. *Am. J. Physiol.*, **193**, 213–218.

LAMMERS, W. & RITCHIE, J. M. (1955). The action of quinine and quinidine on the contractions of striated muscle. *J. Physiol., Lond.*, **129**, 412–423.

MARSHALL, I. G. (1969). The effects of some hemicholinium-like substances on the chick biventer cervicis muscle preparation. *Eur. J. Pharmac.*, **8**, 204–214.

MCEWEN, L. M. (1956). The effect on the isolated rabbit heart of vagal stimulation and its modification by cocaine, hexamethonium and ouabain. *J. Physiol., Lond.*, **131**, 678–689.

PATON, W. D. M. & ZAIMIS, E. J. (1951). The action of d-tubocurarine and of decamethonium on respiratory and other muscles in the cat. *J. Physiol., Lond.*, **112**, 311–331.

PRESTON, J. B. & VAN MAANEN, E. F. (1953). Effects of frequency of stimulation on the paralyzing dose of neuromuscular blocking agents. *J. Pharmac. exp. Ther.*, **107**, 165–171.

SCHUELER, F. W. (1960). The mechanism of action of the hemicholiniums. *Int. Rev. Neurobiol.*, **2**, 77–97.

STOVNER, J. (1957). The effect of low calcium and of tetraethylammonium (T.E.A.) on the rat diaphragm. *Acta physiol. scand.*, **40**, 285–296.

STOVNER, J. (1958). The anticurare activity of tetraethylammonium (T.E.A.). *Acta pharmac. tox.*, **14**, 317–332.

TAKEUCHI, N. (1963). Effects of calcium on the conductance change of the end-plate membrane during the action of transmitter. *J. Physiol., Lond.*, **167**, 141–155.

WISLICKI, L. (1958). Effects of rate of stimulation and of fatigue on the responses to neuromuscular blocking agents. *Br. J. Pharmac. Chemother.*, **13**, 138–143.

(*Received November* 6, 1969)