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NEUROMUSCULAR BLOCKING PROPERTIES OF STEREOISOMERIC ANDROSTANE-3,17-BISQUATERNARY AMMONIUM SALTS

BY

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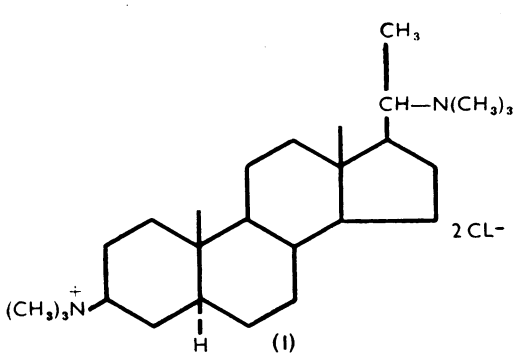
Many attempts have been made to deduce the configuration of a receptor from studies of the structure and activity of agonist or antagonist drugs. One of the most active fields in this respect has been that of the curare-like agents, and several theories have been advanced to explain the structure-activity relationships of these compounds. The potent neuromuscular blocking activity or ganglion blocking activity of many bisquaternary ammonium salts has been envisaged in terms of a two-point attachment of the cationic heads to separate anionic receptor sites on the post-synaptic membrane (Barlow & Ing, 1948a, b ; Paton & Zaimis, 1948, 1949 ; Gill, 1959). Deductions about the spatial separation of these receptor sites have been made from a study of the interonium distances of the polymethylene bisquaternary ammonium salts (Barlow, 1960). In this series, peak neuromuscular blocking activity has usually been observed where the maximum possible interonium separation (fully opposed conformation) is ca 14Å. However, the use of freely flexible molecules in these studies could be misleading, as has been pointed out by Alauddin & Martin-Smith (1962).

The discovery of the curarizing properties of the bisquaternary steroidal alkaloid, malouétine chloride, 3β,20α-bisdimethylamino-5α-pregnane bismethochloride (1) (Quevauviller & Lainé, 1950), prompted the syntheses in our research laboratories of several series of androstane-3,17- and pregnane-3,20-bisquaternary ammonium salts based upon the relatively rigid steroid



(I)

skeleton (Biggs, Davis & Wien, 1964). The use of a steroid nucleus to confer rigidity has been discussed by Alauddin & Martin-Smith (1962) and by Stenlake (1963), and the syntheses and activity of some bisquaternary steroids have been independently reported (Khuong Huu-Lainé & Pinto-Scognamiglio, 1964; Alauddin, Caddy, Lewis, Martin-Smith & Sugrue, 1965).

The results obtained with a bisquaternary 3,17-dipyrrolidin-1′-yl-androstane derivative (dipyrandium chloride, Compound 1, Table 1) led us to examine the potency and duration of action in the cat and monkey of all eight possible stereoisomers (α- and β-epimers at each of positions 3, 5 and 17). We hoped that a study of the properties of these rigid molecules might enable us to distinguish between the various theories advanced to explain the neuromuscular blocking properties of bisquaternary ammonium salts.

METHODS

Cats, of either sex, weighing 1.2–3 kg were used. Anaesthesia was induced with ether and maintained with chloralose (80 mg/kg intravenously). Pointed drill rods were driven through the distal end of the femur and the tibia to enable the leg to be rigidly clamped. The tibialis anterior tendon was cut and attached to a spring myograph writing on a smoked drum. Shielded platinum electrodes were applied to the peripheral end of the crushed sciatic nerve. Twitches of the muscle were elicited by single square wave impulses of 0.1–0.3 msec duration at a supramaximal voltage. Frequency of stimulation was 10 shocks/min. The muscle was kept warm by the use of a small heating lamp. In most experiments blood pressure was recorded from a carotid or femoral artery using a mercury manometer. Drugs were administered through a polythene cannula inserted into a femoral or jugular vein. Artificial respiration was applied throughout the duration of the experiment.

Rhesus monkeys (*Macaca mulatta*) of either sex, weighing 1.8–2.8 kg were used. Anaesthesia was induced either with intravenous thiopentone sodium (20–30 mg/kg) and maintained with chloralose (80 mg/kg intravenously) or induced with pentobarbitone sodium (25–30 mg/kg intravenously) and maintained by further small doses as required. The muscle was prepared and stimulated in the same way as in the cat experiments.

In each experiment a dose of dipyrandium chloride (M&B 9105A) was found which produced approximately 50% blockade. This dose was then repeated until two successive equal blockades were obtained. The potency and duration of action of each compound was compared with dipyrandium in the cat using a 2×2 technique (B.P. 1963), and in the monkey by obtaining randomized dose response curves for each compound.

In addition, the maximum inhibition of the muscle response was measured for each dose and from a plot of % inhibition against log dose of drug, that dose producing 50% inhibition (ED50) was obtained. A plot of time to 100% recovery against % maximal inhibition was made and from this the duration of action at 50% inhibition was determined. This was termed "duration of action."

The mechanism of action of the isomers was determined by reversing the blockade with edrophonium (0.5 mg/kg intravenously) and by injection into day-old chicks. All the stereoisomers were non-depolarizing in these two tests.

The interonium distances (Table 1) were measured from molecular models of the stereoisomers made from Dreiding Stereomodels, assuming that ring A remained in the chair form. Rings B and C are locked in the chair form by fusion with neighbouring rings, while the cyclopentane ring D carrying a 17β-side chain is believed to exist in the "envelope" form (Eliel, Allinger, Angyal & Morrison, 1965a). These measurements do not take into account substituent effects which could slightly distort bond angles and bond lengths possibly resulting in a difference between actual and theoretical interonium distances. It is also possible that the preferred conformation could be influenced by attachment to the substrate.

Drugs

The syntheses of the stereoisomeric quaternary salts are being described in detail elsewhere (Davis, Parnell & Rosenbaum, 1967).

Other drugs used were: α-chloralose (L. Light & Co.); edrophonium (Tensilon, Roche Products Ltd.); gallamine triethiodide (Flaxedil, May & Baker, Ltd.); pentobarbitone sodium (Sagatal, May & Baker, Ltd.); suxamethonium bromide (Brevidil "M," May & Baker, Ltd.); tubocurarine chloride (Burroughs Wellcome Ltd.); thiopentone sodium (Intraval Sodium, May & Baker Ltd.).

RESULTS

Experiments in the cat

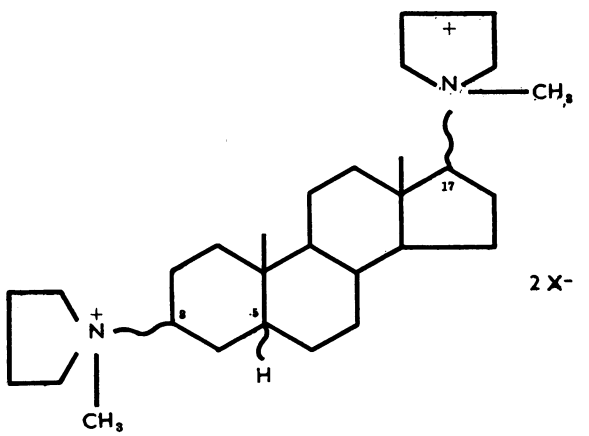
(a) Potency (Table 1)

In each experiment the potency of an isomer was compared with that of dipyrandium, the 3β, 17β, 5α stereoisomer (I). A typical experiment is shown in Fig. 1.

TABLE 1

THE NEUROMUSCULAR BLOCKING ACTION AND APPROXIMATE INTERONIUM DISTANCE OF THE STEREOISOMERIC ANDROSTANE-3,17-BISQUATERNARY AMMONIUM SALTS ON THE SCIATIC NERVE TIBIALIS MUSCLE PREPARATION OF THE CAT OR MONKEY

Figures in brackets refer to the number of experiments performed. Except for suxamethonium, all compounds were non-depolarizing in both species. The mean ED50 (for definition see Methods) for compound I, dipyrandium, was 0·071±0·031 mg/kg (25) in the cat and 0·033±0·013 mg/kg (28) in the monkey. The interonium distances were measured from Dreiding models of the stereoisomers



Compound No.	X	Configuration 3	17	5	Relative molar potency (Dipyrandium=1·00) Cat	Monkey	Interonium distance (Å)
I	Cl	β	β	α	1·00	1·00	11·0
II	I	β	α	α	1·00 (2)	0·92 (2)	10·6
III	I	α	β	α	0·56 (3)	0·86 (1)	10·4
IV	I	α	α	α	0·43 (4)	0·76 (2)	9·6
V	I	β	β	β	0·91 (2)	1·25 (1)	10·0
VI	I	β	α	β	0·67 (3)	0·79 (1)	9·3
VII	I	α	β	β	0·09 (3)	0·08 (3)	9·9
VIII	I	α	α	β	0·09 (3)	0·07 (3)	8·7
d-Tubocurarine	Cl	–	–	–	1·31 (3)	1·53 (2)	—
Gallamine	I	–	–	–	0·19 (12)	0·33 (7)	—
Suxamethonium	Br	–	–	–	1·26 (4)	0·08 (3)	—

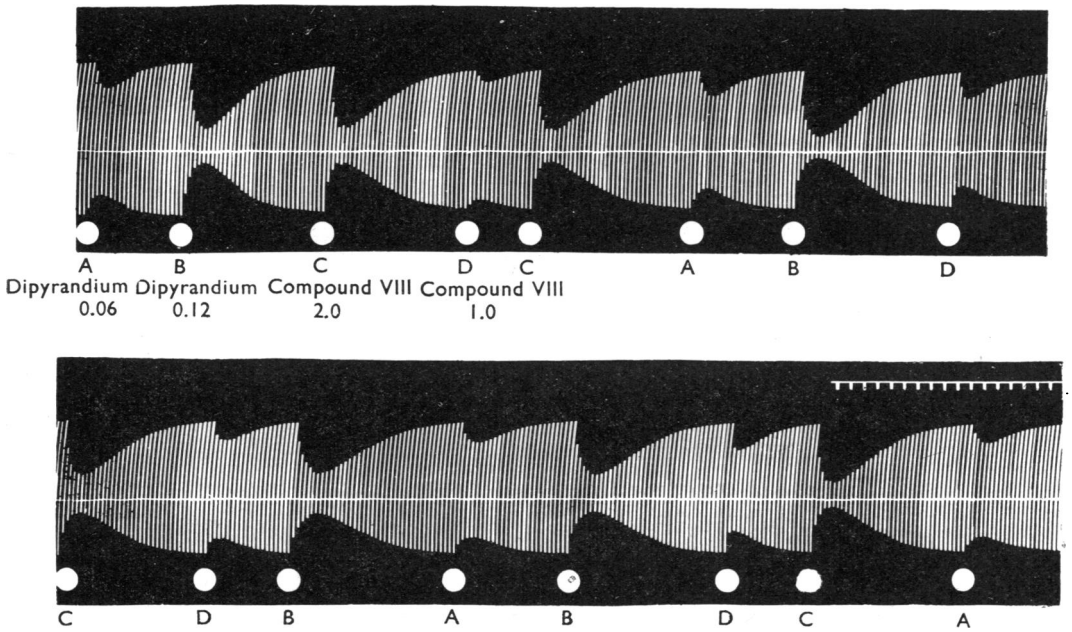


Fig. 1. Cat sciatic nerve tibialis muscle preparation. A comparison of the potency of Compound VIII with dipyrandium. Doses of dipyrandium or Compound VIII in the quantities indicated (mg/kg of the appropriate salt) were injected intravenously at the marks. Dipyrandium was approximately 11 times more potent than Compound VIII in this experiment. Time marker every 30 sec.

In the 5α series, the 3β, 17β (I) and the 3β, 17α (II) isomers were equipotent, but the 3α, 17β (III) and 3α, 17α (IV) isomers had half the potency of I or II.

In the 5β series, the 3β, 17β (V) was the most potent isomer, and slightly less active than dipyrandium. The 3β, 17α isomer (VI) had three-quarters of the potency of V, but the 3α, 17β (VII) and the 3α, 17α (VIII) isomers had less than one-tenth of the potency of V.

Dipyrandium itself was slightly less potent than d-tubocurarine or suxamethonium, but about five times as potent as gallamine.

The blockade produced by all the compounds could be readily reversed by injection of edrophonium (0.5 mg/kg intravenously) (Fig. 2a).

(b) Duration of action (Table 2)

The duration of action of all the isomers were not significantly different from that of dipyrandium. The durations of action of dipyrandium and suxamethonium were approximately equal in this species and about one-third that of gallamine and one-quarter that of tubocurarine.

Experiments in the monkey

(a) Potency (Table 1)

Again, the potency of each stereoisomer was compared with that of dipyrandium (I). In most cases the result is based on only one experiment, though for the least potent

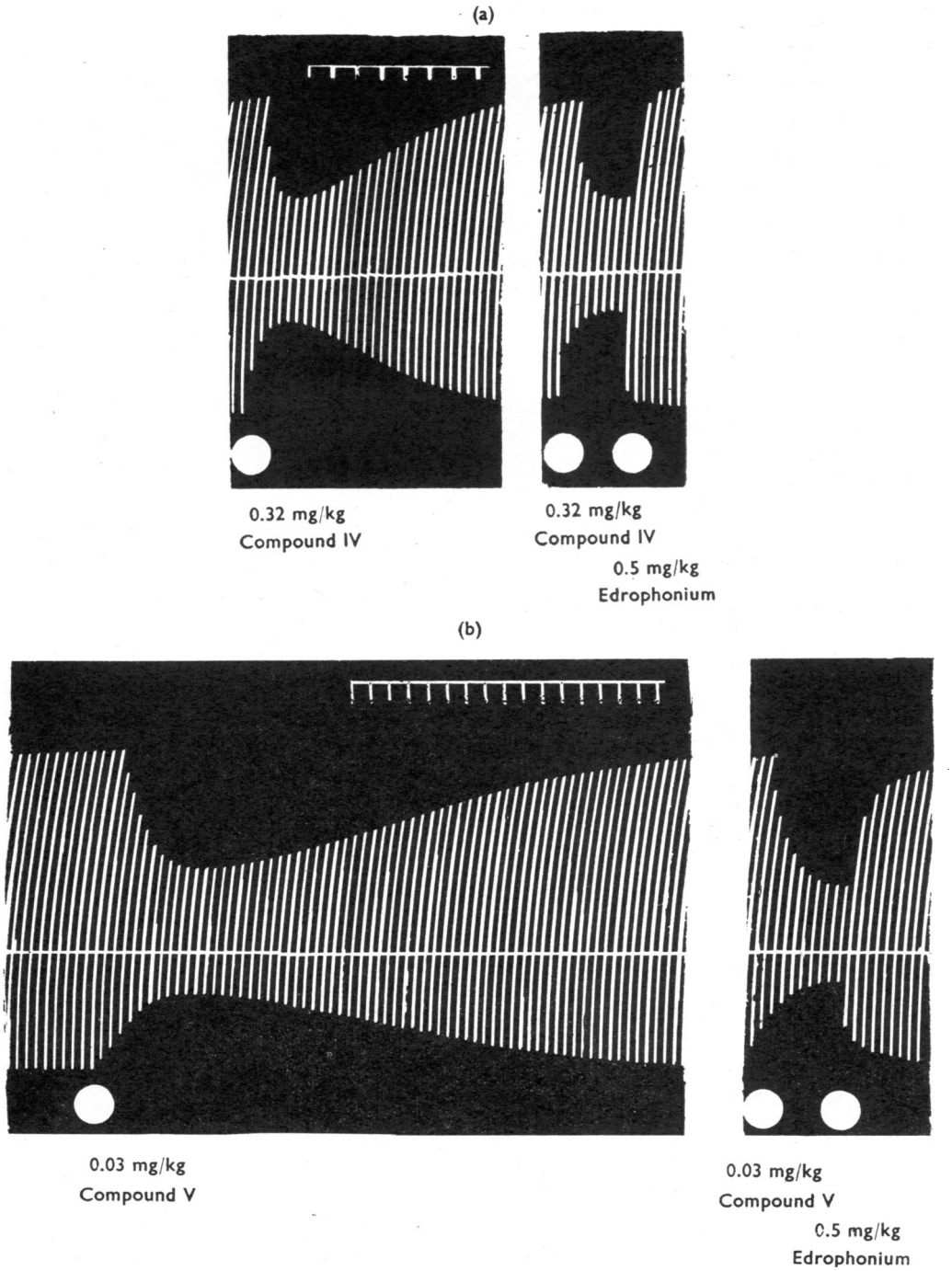


Fig. 2. (a) Cat sciatic nerve tibialis preparation. The record shows the reversal of blockade produced by Compound IV by edrophonium (0.5 mg/kg). Drugs were injected at the time marks, time marker every 30 sec. (b) Monkey sciatic nerve tibialis preparation. The record of the reversal of blockade produced by Compound V by edrophonium (0.5 mg/kg). Drugs were injected at the marks, time marker every 30 sec. Note the longer duration of the isomers in the monkey.

TABLE 2

THE DURATION OF ACTION AT THE ED50 OF DIPYRANDIUM, TUBOCURARINE, GALLAMINE OR SUXAMETHONIUM ON THE CAT OR MONKEY SCIATIC NERVE TIBIALIS MUSCLE PREPARATION

Figures in brackets refer to the number of experiments performed

	Cat		Monkey	
Compound	Duration of action in min at the ED50	Relative duration of action (Dipyrandium = 1·00)	Duration of action in min at the ED50	Relative duration of action (Dipyrandium = 1·00)
Dipyrandium	5·0 (25)	1·00	13·0 (28)	1·00
d-Tubocurarine	20·5 (3)	4·10	24·1 (3)	1·86
Gallamine	14·8 (12)	2·96	13·7 (7)	1·05
Suxamethonium	5·2 (4)	1·04	4·1 (3)	0·32

isomers three experiments were performed. The relative potencies of the isomers in both the 5α and 5β series parallel the results obtained in the cat, but all the isomers were approximately twice as potent in the monkey as in the cat. Again, the block produced by the isomers could be reversed by edrophonium (0.5 mg/kg intravenously) (Fig. 2b).

(b) Duration of action (Table 2)

The duration of action of each isomer was not significantly different from that of dipyrandium in the same experiment. Further, in the monkey, dipyrandium was not significantly shorter acting than gallamine. It was two to three times longer acting in the monkey than in the cat (Fig. 2).

DISCUSSION

There has been some speculation about the neuromuscular blocking properties of bisquaternary steroids (Alauddin & Martin-Smith, 1962; Alauddin, Caddy, Lewis, Martin-Smith & Sugrue, 1965), and the effects of several compounds have been described in detail (Quevauviller & Lainé, 1960; Khuong Huu-Lainé & Pinto-Scognamiglio, 1964; Biggs, Davis & Wien, 1964; Alauddin *et al.*, 1965). However, little work has been published on the influence of stereoisomerism on the properties of 3,17-bisquaternary steroids. We have studied eight 3,17-bisquaternary androstane derivatives forming a complete series of all possible stereoisomers at positions 3, 5 and 17. All four 5α-androstanes studied were very active, but the 3α-isomers were 1.5–2.3 times less potent than the corresponding 3β-compounds. These results agree well with those of Khuong Huu-Lainé & Pinto-Scognamiglio (1964) who synthesized and studied a series of 5α-pregnanes based on the alkaloid malouétine. They observed that there was little difference in potency within their series, but that the 3α-isomers were 1.3 times less potent than the corresponding 3β-compounds. These workers did not prepare or study any of the 5β-isomers.

In the 5β-androstane series the 3α-members were approximately ten times less potent than the corresponding 3β-members and the 3β-members were 1.1–1.4 times less potent

than the most active members of the 5α-series. Thus, in both 5α- and 5β-series, the most potent members possessed the 3β-configuration and the least potent members possessed the 3α-configuration.

In contrast, inversion of the basic centre at the 17-position had little effect on potency in either the 5α- or the 5β-series, even when the two quaternary heads were on opposite sides of the ring system and so hindered by the rigid skeleton that simultaneous attachment of both to receptors seemed unlikely.

Interonium distance in bisquaternary salts has been considered to be an important factor in determining potency (Paton & Zaimis, 1948, 1949 ; Barlow & Ing, 1948a, b). We find no such general relationship between potency and interonium distance in these steroid derivatives. Thus compounds V and VII, with a similar interonium distance, showed a tenfold difference in potency and compounds VII and VIII, where there was an interonium distance difference of 1.2 Å, were equipotent. This would suggest that the configuration of the quaternary centres, and the shape and nature of the structure joining them, are at least as important as interonium distance in determining neuromuscular blocking potency.

The relatively rigid bisquaternary steroids cannot fold so that both quaternary heads could simultaneously interact with a single anionic centre in the manner postulated by Cavallito & Gray (1960) for flexible bisquaternary ammonium salts, following the conductimetric experiments on the stability of such ion pairs reported by Brody & Fuoss (1956). If we consider that the most potent stereoisomers were always the 3β-bisquaternary members of each series, that change in this configuration causes a loss of potency of two to eleven times, and further that configurational change at the 17-position does not significantly affect potency, then one might postulate that interaction with the receptor at the 3-position is more important than interaction in either configuration at the 17-position. Thus our results would tend to support the adumbration theory of Loewe & Harvey (1952), who postulated a " one point " attachment theory, where the bulk of the molecule, in this case presumably the steroid nucleus, shields the receptor, rather than the suggestion of Cavallito & Gray (1960), and Waser (1959), that a two point receptor complex could be formed. The relatively flat steroid nucleus in the 5α-series may be a more effective shield than the more folded steroid nucleus of the 5β-series, and therefore the 5α-series should be more potent than the corresponding members of the 5β-series, which accords with our observations.

Lonsdale, Milledge & Pant (1965) suggested that in the bisquaternary polymethylene series neuromuscular blocking potency is dependent not on interonium distance, but on the separation of the extreme methyl groups on either side of the polymethylene chain, and on the number of possible van der Waal contacts within these extremes. In our series one would expect that the more planar 5α-androstanes would form a large number of such van der Waal bonds. This again might account to some extent for the difference in potency between the 5α- and 5β-series, though there remains the possibility of an edge-on attachment of the steroid nucleus to the receptor (Alauddin *et al.*, 1965), and throughout we have neglected the possibility of chair to boat conformational isomerism in ring A. However, it is accepted that ring A normally exists in the chair form unless a severe 1,3-diaxial repulsion occurs which can be relieved by going over to the boat form (Eliel, Allinger, Angyal & Morrison, 1965b).

The duration of action of the stereoisomers did not differ significantly from that of dipyrandium in either species. We have confirmed and extended the observations of Biggs *et al.* (1964) on the difference between the duration of action of dipyrandium in cat and monkey, and have found that it is nearly three times longer acting in the monkey than in the cat. Thus the duration of action of dipyrandium in the monkey resembles that in man (Mushin & Mapleson, 1964) and is far longer than in the cat, dog, rabbit or chicken.

SUMMARY

1. The neuromuscular blocking activity and duration of effect of eight stereoisomeric androstane-3,17-bisquaternary salts have been studied *in vivo* on the cat or monkey sciatic nerve tibialis muscle preparation.

2. The 3β-isomers were in general more potent than the corresponding 3α-compounds in both the cat and monkey.

3. All the stereoisomers were twice as potent, and nearly three times longer acting in the monkey than in the cat.

4. It was concluded that there was no general relationship between interonium distance and potency in this group of steroid derivatives.

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