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Neuromuscular and other blocking actions of a new series of mono and bisquaternary aza steroids*

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The effects of a newly-synthesized series of mono and bisquaternary aza steroids have been investigated in the anaesthetized cat. All the compounds have been tested for neuromuscular blocking, ganglion blocking and vagolytic activity. The monoquaternary compounds of the series were weak neuromuscular blocking agents, their main action being to produce ganglion blockade and a fall in blood pressure. Both the bisquaternary compounds of the series primarily exhibited neuromuscular blocking activity of short duration and rapid onset. 4-Methyl-17β-dimethylamino-4-aza-5α-androstane dimethiodide (HS-467) was approximately equipotent with tubocurarine as a neuromuscular blocking agent, whereas 17a-methyl-3β-pyrrolidino-17a-aza-D-homo-5-androstene dimethiodide (HS-310, chandonium iodide) was 4–5 times more active. Both compounds exhibited extremely weak ganglion blocking activity. Structure-activity relations are discussed, and it is concluded that in this series two quaternary heads are necessary for optimal neuromuscular blocking activity. The possibility that in the bisquaternary compounds inter-onium distance, or stereochemical conformation may be the main factor in determining specific neuromuscular blocking activity is discussed.

Many mono and bisquaternary steroidal compounds have been shown to antagonize the nicotinic actions of acetylcholine both at the neuromuscular junction (see Martin-Smith, 1971; Buckett, 1972 for reviews) and at autonomic ganglia (Marshall & Martin-Smith, 1972). One bisquaternary compound, pancuronium, is currently in widespread clinical use as a muscle relaxant.

Recently, the chemically novel compound 4, 17a-dimethyl-4,17a-diaza-D-homo-5α-androstane dimethiodide (HS-342; I), in which the quaternary groups are incorporated into the steroid nucleus, has been synthesized (Singh, Paul & Parashar, 1972, 1973) and tested for potential neuromuscular blocking activity (Marshall, Paul & Singh, 1973a,b). This compound possesses an inter-onium distance of about 8Å and exhibits both neuromuscular blocking activity of high potency and short duration, together with some ganglion blocking activity.

The results now presented concern the pharmacological actions of a series of structural analogues of HS-342 (see Fig. 1) (Singh & Paul, 1974) synthesized to assess

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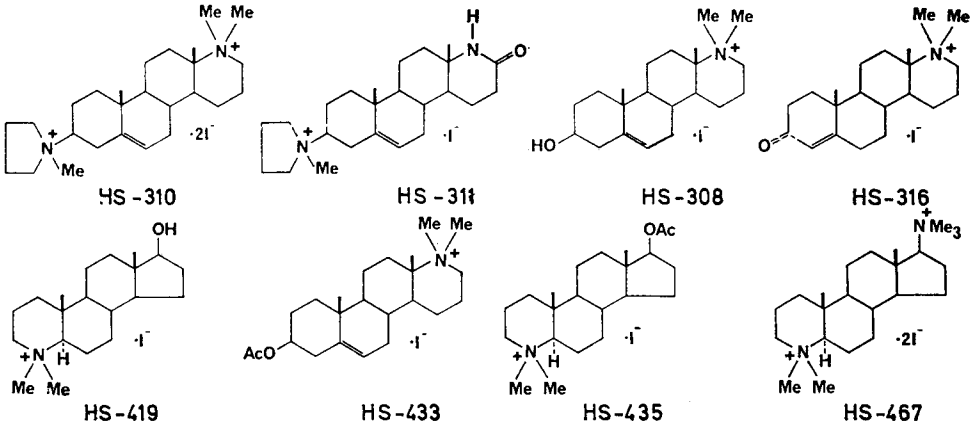
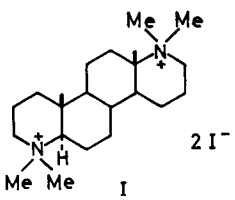


FIG. 1. Chemical formulae of the test compounds.

the importance of the quaternary groups and inter-onium distance on neuromuscular and ganglion blocking activity.

Some of these results have been presented to the Indian Pharmaceutical Congress Association at New Delhi in December, 1973 (Gandiha, Marshall & others, 1973).



I

METHODS

General

All experiments were carried out on cats of either sex anaesthetized with a mixture of α-chloralose (8 ml kg^{-1} of a 1 % solution) and pentobarbitone sodium (2·5 mg kg^{-1}) injected intraperitoneally. The cats were artificially ventilated (15–20 ml of air kg^{-1}) throughout the experiments. Blood pressure was recorded from either a common carotid or a femoral artery by a polythene cannula attached to a Statham P23Ac pressure transducer. Drugs were injected into either an external jugular or a brachial vein via a polythene cannula.

Neuromuscular junction

The sciatic nerve was exposed in the popliteal space, ligated and crushed centrally to the ligature. The nerve was stimulated through a stimulus isolation unit by bipolar platinum electrodes,with rectangular pulses of 0·1 ms duration and of sufficient strength to elicit maximal twitches of the tibialis anterior and soleus muscles. One hind limb was immobilized by drills placed through the lower ends of the femur and tibia. Isometric tension changes of the tibialis anterior and soleus muscles in response to stimulation of the sciatic nerve, at a frequency of 0·1 Hz, were recorded using Grass FT10C and FT03C force displacement transducers respectively.

Nictitating membrane

The left cervical nerve was separated from the vagus nerve, ligated and stimulated centrally to the superior cervical ganglion, through bipolar platinum electrodes placed peripherally to the ligation. The nerve was stimulated by trains of rectangular pulses of 0·5 ms duration at a frequency of 20 Hz, and of sufficient strength to elicit a maximal contraction of the nictitating membrane. The train duration was 10 s and the train frequency was 0·01 Hz. Isometric tension changes in the nictitating membrane were recorded by Grass FT03C force displacement transducers.

Cardiac vagus neuroeffector junction

In some experiments the left vagus nerve was separated from the cervical nerve, ligated and periodically stimulated by 10 s trains of rectangular pulses (0·5 ms duration, 20 Hz frequency). Heart rate was measured from the blood pressure recording, using a fast paper speed.

RESULTS

In anaesthetized cats all the test compounds were assessed for neuromuscular blockade, ganglion blocking and vagolytic activity. The ED50's for these effects are shown in Table 1. From these results it can be seen that all the compounds tested possess, to varying degrees, the above properties. In common with other quaternary compounds acting at the neuromuscular junction, the effect seen at the lowest doses with both the mono and bisquaternary compounds was the vagolytic action, although this effect did not appear to be important in determining the blood pressure responses observed. The effects of drugs on the blood pressure appeared to parallel the degrees of ganglion blocking activity possessed by individual compounds.

Table 1. *ED50 values (mg kg^{-1}) for vagolytic action and ganglion and neuromuscular blocking actions in the anaesthetized cat.*

Compound	Vagus	Nictitating membrane	Tibialis anterior	Soleus
HS-308	0·7 (2·14)	0·55 (2·7)	1·5	2·2 (0·68)
HS-310	0·04 (1·75)	1·0 (0·07)	0·07	0·06 (1·16)
HS-311	0·5 (19·0)	9·0 (1·05)	9·5	10·0 (0·95)
HS-316	0·09 (16·7)	0·5 (3·0)	1·5	2·0 (0·75)
HS-419	0·35 (7·14)	0·5 (5·0)	2·5	3·0 (0·83)
HS-433	0·18 (27·8)	0·7 (7·14)	5·0	5·2 (0·96)
HS-435	0·45 (5·0)	1·25 (1·8)	2·25	3·0 (0·75)
HS-467	0·19 (1·7)	3·0 (0·1)	0·325	0·375 (0·87)
HS-342	0·1 (2·5)	1·5 (0·17)	0·25	0·3 (0·8)

Figures in parentheses indicate activity of compounds relative to their activity on the tibialis anterior muscle (1·0).
ED50 values represent the mean of between 3 and 6 separate experiments.

Both the bisquaternary ammonium compounds exhibited powerful neuromuscular blocking activity with little ganglion blocking activity (Table 1). Compound HS-467 was slightly less powerful as a neuromuscular blocking drug than the parent compound HS-342, and, relative to its neuromuscular blocking activity, showed rather less ganglion blocking activity than did HS-342. The vagolytic action of this com-

pound was also relatively weak and the overall result on the cardiovascular system was a slight fall in blood pressure (Fig. 2A). Dose-response curves for the effects of HS-467 are shown in Fig. 2B.

Compound HS-310 exhibited powerful neuromuscular blocking activity, being 3·5–5 times more potent than HS-342 and (+)-tubocurarine, and, like HS-342, having a short duration and rapid onset. In addition, the ganglion blocking activity of HS-310 was extremely weak and the vagolytic action appears to be the factor in producing a slight tachycardia. This compound was potentially the most interesting of the series and has been assigned the name chandonium iodide.

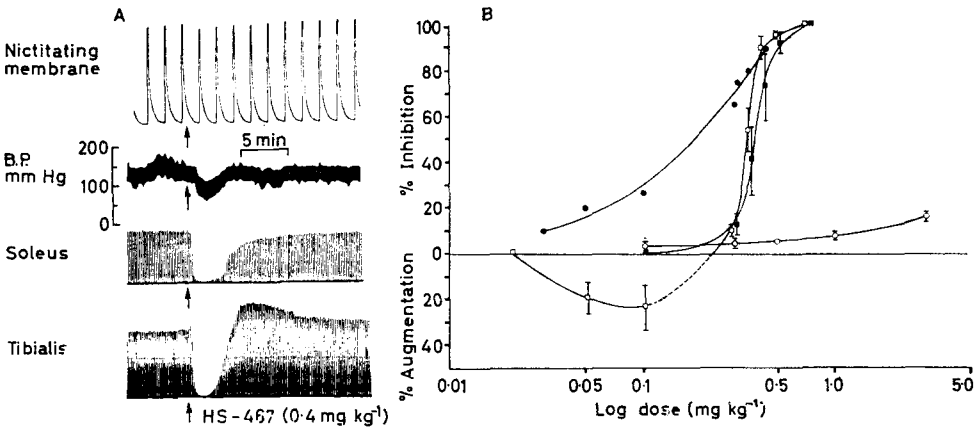


FIG. 2 A. Chloralose-anaesthetized cat. The effects of a neuromuscular blocking dose of HS-467 on the preganglionically stimulated nictitating membrane, the arterial blood pressure and the soleus and tibialis anterior muscles. Although the predominant effect is on the skeletal muscles, note the slight depression of the responses of the nictitating membrane, and the fall in blood pressure.

B. Dose-response curves for HS-467 on the responses of the heart rate to vagal stimulation (●——●), of the nictitating membrane to preganglionic nerve stimulation (○——○), and of the tibialis anterior (□——□) and soleus (■——■) muscles to single shock stimulation of the sciatic nerve. Each point represents the mean of at least three determinations.

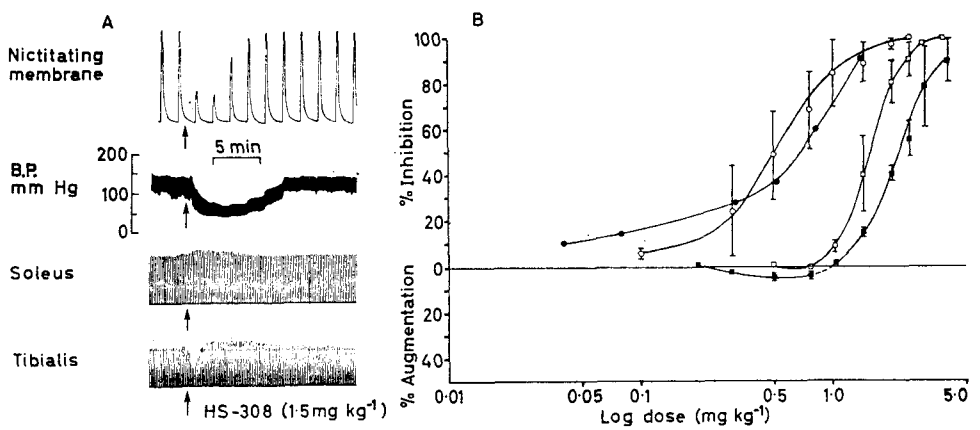


FIG. 3 A. Chloralose-anaesthetized cat as in Fig. 2. The effects of HS-308. Note the weak and short-lived neuromuscular blocking activity of HS-308, and the more pronounced and longer-lasting actions on the nictitating membrane and the blood pressure.

B. Dose-response curves for HS-308 as in Fig. 2B.

In contrast, all the monoquaternary compounds of the series exhibited very weak neuromuscular blocking activity, the most potent of the monoquaternaries (i.e. HS-308 and HS-316) being some six times less active than HS-342. The predominant nicotinic blocking action of these compounds was at autonomic ganglia, with the result that the compounds produced a fall in blood pressure (Fig. 3A). The most potent ganglion blocking drugs of the series were compounds HS-308, HS-316, HS-419 and HS-433; these possessed ratios greater than 2·5 of ganglion blocking to neuromuscular blocking activity (Table 1), indicating a prominence of the former activity. These compounds were equipotent with hexamethonium as ganglion blocking drugs. Dose-response curves for the effects of HS-308 and HS-433 are shown in Figs 3B and 4 respectively. Compound HS-311 possessed extremely weak blocking activities at all the sites examined.

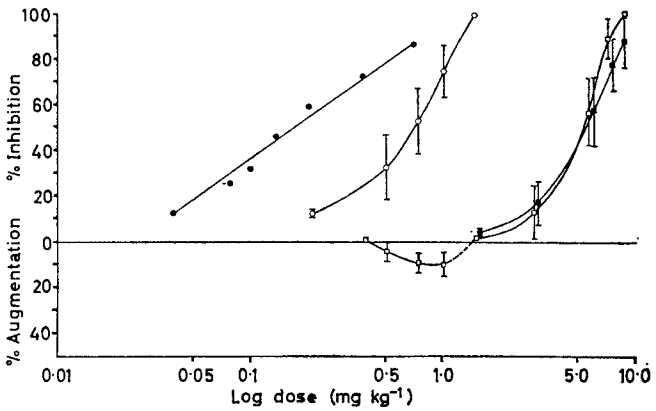


FIG. 4. Dose-response curves for HS-433 as in Fig. 2B.

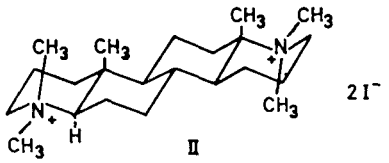
The neuromuscular block produced by all the compounds of the series appeared to be of the non-depolarizing nature, as evidenced by their reversibility by anticholinesterase agents and the lack of muscle fasciculations. Most of the compounds exhibited a short-lived degree of twitch augmentation in the tibialis anterior muscle, particularly at sub-neuromuscular blocking doses, and subsequent to the neuromuscular block.

DISCUSSION

In bisquaternary ammonium compounds it is generally accepted that the optimal inter-onium distance for neuromuscular blocking activity is in the region of 10–12 Å. Ganglion blocking activity does not appear to be so dependent upon inter-onium distance, but as the inter-onium distance falls below 10 Å, so the neuromuscular blocking activity diminishes, with the result that ganglion blocking activity becomes more predominant (Bowman & Webb, 1972). In compounds possessing inter-onium distances of around 6·5 Å, neuromuscular blocking activity is almost entirely absent (Gill, 1959; Marshall & Martin-Smith, 1972).

4,17a-Dimethyl-4,17a-diaza-D-homo-5α-androstane dimethiodide (HS-342) possessed the inter-onium distance of around 8 Å as measured with Dreiding models. The quaternary nitrogens are present in a rigid setting, with steric crowding manifested

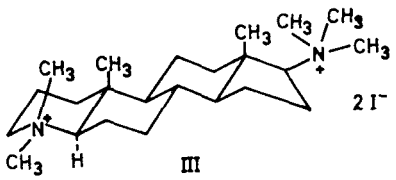
particularly on the β face of the molecule (II). The planar and less hindered α side is expected to be involved in interaction with the bioreceptor.



II

In the anaesthetized cat HS-342 exhibited both non-depolarizing neuromuscular and ganglion blocking activity (Marshall & others, 1973 a, b), and the inter-onium distance may be the determining factor for this mixture of activities. Despite the ganglion blocking activity observed, and the concomitant slight fall of blood pressure, HS-342 was a potent muscle relaxant, approximately equal in potency to (+)-tubocurarine, but with an onset and duration of action similar to that of the recently introduced short-acting drug AH 8165, 1,1-azobis[3-methyl-2-phenyl-1H-imidazo-(1,2-a)pyridinium dibromide (Marshall & others, 1973b).

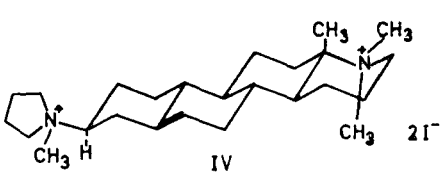
4-Methyl-17β-dimethylamino-4-aza-5α-androstane dimethiodide (HS-467) has the inter-onium distance (9·0Å) somewhat extended (III) as compared to HS-342 (II),



III

and the 17β quaternary ammonium function may have a little better freedom for interaction. It was found to be only slightly less active than HS-342 as a neuromuscular blocker and vagolytic agent, but possessed considerably less ganglion blocking activity than HS-342. This separation of the activities was even more evident in 17a-methyl-3β-pyrrolidino-17a-aza-D-homo-5-androstene dimethiodide (chandonium iodide) (HS-310), which showed a dramatic increase in neuromuscular blocking activity over HS-342, with virtually no ganglion blocking action. Although this compound is not as potent as pancuronium, it possessed a short duration of action in the cat, and appears to be the most potent short-acting non-depolarizing drug reported so far in the literature.

Chandonium iodide (HS-310) in its conformational perspective (IV) indicates not only a sufficient increase in the inter-onium distance (10·2Å) but also has one of the quaternary functions well exposed (3β-equatorial). Further, if through pyrrolidine we visualize the backs of two alkyl groups tied off, this function may also be of



IV

added steric advantage. The 5,6-double bond will affect the geometry of ring B and also of ring A. Whether the degree of receptor selectivity in chandonium iodide can be accounted for through only the increase in inter-onium distance relative to HS-342 or whether the steric features are also the determining factors cannot be said with certainty.

It has been postulated that, in neuromuscular blocking drugs, binding to acetylcholine receptors may occur at only one of the cationic heads, with the rest of the bulky molecule shielding the receptors from the neurally released acetylcholine (Loewe & Harvey, 1952). As such it was considered of interest to examine the related mono-quaternary compounds.

HS-308, HS-316 and HS-433 have quaternary nitrogen functions at position 17a and polar groups at position 3, at more or less the same distance from the 17a cation as the 3β-pyrrolidino in chandonium iodide. HS-311 possesses the 3β cationic character similar to that of chandonium iodide and the ring D has a polar lactam function. Correspondingly, HS-419 and HS-435 have quaternary functions at position 4 and polar functions at the 17β position, at nearly the same distance from position 4 as the 17β cation in HS-467.

These monoquaternary compounds exhibited extremely weak neuromuscular blocking action, although several of them were equal in potency to hexamethonium as ganglion blocking agents. Thus, it appears that both the cationic heads are necessary for neuromuscular blocking activity. It is possible, however, that in bis-quaternary compounds one of the onium heads binds tightly to the receptor surface, the bulky molecule shields the receptor and the second onium head provides a rather looser anchoring force.

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