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The neuromuscular and autonomic blocking effects of azasteroids containing choline or acetylcholine fragments

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Nine analogues of the azasteroid muscle relaxant chandonium have been tested for neuromuscular blocking activity, ganglion blocking activity, inhibitory effects on cardiac muscarinic receptors, and for effects on noradrenergic transmission. Experiments were performed in anaesthetized cats and in isolated preparations. The two bisquaternary compounds HS-626 and HS-627, choline and acetylcholine-like analogues of chandonium respectively, were approximately equipotent with chandonium as neuromuscular blocking agents in the cat, but HS-672 possessed slightly more vagal blocking action. No evidence was obtained for ganglion block. The monoquaternary analogues, HS-408 and HS-465 and two 4-aza-androstanes, (HS-522 and HS-523) were more than 100 times less active than the bisquaternary compounds as neuromuscular blocking agents, and produced vagal blockade and ganglion blockade at sub-neuromuscular blocking doses. pA_2 determinations in the chick biventer cervicis muscle, the guinea-pig atria and guinea-pig ileum showed that the bisquaternary compounds chandonium, HS-626 and HS-627 were much more potent in blocking cardiac than intestinal muscarinic receptors and that HS-626 possessed the widest margin between concentrations blocking the nicotinic receptors at the neuromuscular junction and the cardiac muscarinic receptors. Evidence for the three bisquaternary compounds blocking neuronal noradrenaline reuptake was obtained only at very high concentrations. HS-626 possessed a slightly more desirable spectrum of activities than chandonium, but the degree of improvement over the parent compound is insufficient to merit extensive clinical testing.

Many mono and bisquaternary androstane compounds have neuromuscular blocking activity and one of these compounds, pancuronium, is presently in widespread use as a muscle relaxant. Pancuronium is approximately ten times more potent than tubocurarine as a neuromuscular blocking agent and its high potency has been ascribed to the presence of acetylcholine-like moieties in the molecule (Buckett et al 1973; Durant et al 1979).

Work in our laboratories has been concerned with the synthesis and pharmacology of a series of quaternary azasteroids, the most potent compound of which, chandonium iodide (17a-methyl-3β-pyrrolidino-17a-aza-D-homo-5-androstene dimethiodide) (HS-310) has a neuromuscular blocking potency in the cat of around one-half of that of pancuronium (Gandiha et al 1974, 1975). Chandonium has negligible ganglion blocking activity but exhibits a blocking action at the cardiac vagus neuroeffector junction at neuromuscular blocking doses (Gandiha et al 1975). This vagal blocking activity, like that of pancuronium, is likely to be associated with the production of tachycardia in anaesthetized man. However, in addition to its high potency, chandonium has a short duration of action in most experimental animals and preliminary work indicates that it also has a shorter duration of action than pancuronium in man (S. Agoston—personal communication).

We now report the pharmacology of four analogues of chandonium containing either choline or acetylcholine moieties (Fig. 1), synthesized in an attempt to confer a greater specificity for the nicotinic acetylcholine receptor at the neuromuscular junction (Singh et al 1979.)

We have also studied the effects of five choline- or acetylcholine- containing androstanes and cholestanes with 4-aza modification as in the neuromuscular blocking azasteroid HS-342 (Marshall et al 1973) (Fig. 1). Since these monoquaternary compounds were relatively inactive as neuromuscular blockers they were not studied in depth.

A preliminary report on some of the compounds was presented at the Vth European Congress of Anaesthesiology, Paris, September, 1978.

METHODS

Experiments in anaesthetized cats

General. Cats of either sex were anaesthetized with a mixture of α-choralose (80 mg kg^{-1}) and pento-

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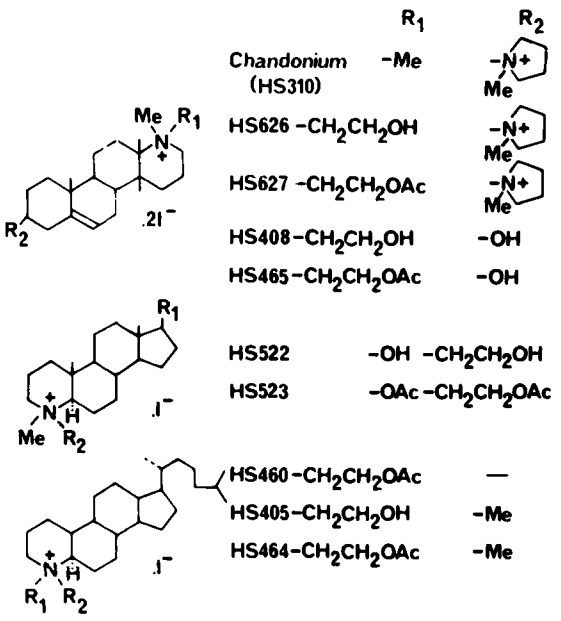

FIG. 1. Chemical formulae of chandonium (HS-310) and test compounds.

barbitone sodium (5 mg kg^{-1}) and were artificially ventilated (18 ml air kg^{-1} at a rate of 26 breaths min^{-1}) throughout the experiments. Arterial blood pressure was recorded from the femoral artery by a Statham P23Ac pressure transducer. The blood pressure pulse triggered a cardiotachometer (Grass 7P4F) to record heart rate. Drugs were injected into a femoral vein.

Nerve-skeletal muscle preparations. The sciatic nerve was stimulated at a frequency of 0·1 Hz with rectangular pulses of 0·2 ms duration and of strength greater than that required to produce maximal twitches of the tibialis anterior and soleus muscles. Muscle twitches were recorded by Grass FT10C force-displacement transducers.

The time of onset of action of the compounds was measured from injection to 80–99% depression of the maximal twitch. The duration of action of the compounds was measured from injection to the point of recovery to 95% of control twitch height. The time of recovery was measured as the time taken to recover from 25 to 75% of the control twitch height.

Nictitating membrane preparation. The left cervical nerve was ligated and stimulated preganglionically at a point peripheral to the ligation every 100 s with trains of rectangular pulses (0·5 ms duration, 10 Hz frequency for 10 s). Contractions of the nictitating membrane were recorded by Grass FT03C force-displacement transducers.

Vagal nerve stimulation. The right vagus nerve was ligated and stimulated every 100 s with trains of rectangular pulses (0·5 ms duration, 10 Hz frequency for 10 s). The stimulation strength was adjusted to produce a decrease in heart rate of approximately 50% of control heart rate.

Isolated preparations

General. All isolated preparations were bathed in Krebs-Henseleit solution of the following composition (mM): NaCl 118; KCl 4·7; $NaHCO_3$ 26; KH_2PO_4 1·2; $MgSO_4$. $7H_2O$ 1·2; $CaCl_2$ 2·5; dextrose 11. The solutions were maintained at 32 °C and were bubbled with a mixture of 95% oxygen and 5% carbon dioxide. The limited amount of the test drugs did not allow us to construct full dose-response curves to agonists in the presence of the antagonists. Instead, control responses were obtained to a concentration of carbachol that gave a response about 50% of the maximal response and then the response to twice that carbachol concentration was determined in the presence of at least 3 concentrations of antagonist. pA_2 values were calculated for each preparation.

Chick biventer cervicis muscle preparation. The nerve within the muscle tendon was stimulated at a frequency of 0·1 Hz with rectangular pulses of 0·2 ms duration and of strength greater than that required to produce maximal muscle twitches. Periodically nerve stimulation was stopped and acetylcholine ($5 \times 10^{-5} - 10^{-4}$ M) sufficient to produce a contracture approximately equal in amplitude to a maximal muscle twitch was added to the bath and allowed to remain in contact with the tissue for 30 s before washout.

The pA_2 values against carbachol of the two most potent compounds (HS-626 and HS-627) were compared with that of chandonium. Carbachol was allowed to remain in contact with the tissue for 90 s before washout. Antagonist drugs were allowed to remain in contact with the tissue for 5 min before additions of carbachol.

Guinea-pig ileum preparation. The pA_2 values against acetylcholine of the two most potent neuromuscular blocking compounds (HS-626 and HS-627) were compared with that of chandonium.

Guinea-pig atrium preparation. Left atrium preparations were electrically driven at a frequency of 2 Hz by rectangular pulses of 2 ms duration and

of strength sufficient to produce maximum contractions.

pA_2 values of HS-626, HS-627 and chandonium against the negative inotropic effect of acetylcholine were determined in atria removed from guinea-pigs that had been injected with reserpine (1 mg kg^{-1} i.p.) 24 h previously. The preparations were bathed in Krebs-Henseleit solution containing hexamethonium (6×10^{-5} M).

The effects of the above three azasteroids were also studied on the positive inotropic effects of noradrenaline in atria removed from non-reserpinized guinea-pigs and bathed in Krebs-Henseleit solution containing atropine (2×10^{-6} M).

Rat vas deferens preparation. Vasa deferentia bathed in Krebs-Henseleit solution containing atropine (2×10^{-6} M) and hexamethonium (6×10^{-5} M) were stimulated every 10 min with trains of impulses (0·5 ms duration, 20 Hz frequency for 10 s) applied through ring electrodes placed around the mid-portion of the vas deferens. Periodically noradrenaline ($3{\cdot}75 \times 10^{-7}$ M) was added to the tissue bath for 30 s. Augmenting effects of drugs were measured on the second phase of the biphasic response to nerve stimulation.

Drugs and materials

The drugs used were acetylcholine chloride, atropine sulphate, carbachol chloride, α-chloralose, noradrenaline hydrochloride, reserpine, (all Sigma): hexamethonium bromide (Koch-Light); pentobarbitone sodium (Abbott); cocaine hydrochloride (MacFarlan Smith). All drugs were dissolved in 0·9% sodium chloride solution except HS-405, HS-460 and HS-464 which were dissolved in a 50% solution of ethanol. Doses quoted in the text refer to the appropriate salt. The synthesis of the HS compounds is described by Singh et al (1979).

RESULTS

Anaesthetized cat experiments

General. All nine compounds were tested in anaesthetized cats. In one series of experiments dose-inhibition curves for neuromuscular block, vagal block and ganglion block were constructed for each of the compounds. In a second series of experiments the two most potent androstene compounds, HS-626 and HS-627 and their monoquaternary analogues, HS-408 and HS-465, were compared with chandonium in the same cat.

17a-Aza-D-homoandrost-5-enes (HS-626, HS-627, HS-408 and HS-465). When dose-inhibition curves were constructed in individual cats only the bis-quaternary compounds, HS-626 and HS-627 exhibited neuromuscular blocking activity at doses (30–100 μg kg^{-1}) below those producing either vagal or ganglion block (Figs 2, 3). In contrast their monoquaternary analogues, HS-408 and HS-465, exhibited vagal and ganglion blocking activities at doses (25–1000 μg kg^{-1}) below those producing neuromuscular block (2–4 mg kg^{-1}). In the case of HS-465 no neuromuscular block was seen at doses up to 4 mg kg^{-1} (Figs 4, 5). The doses of the compounds producing 50% neuromuscular, vagal and

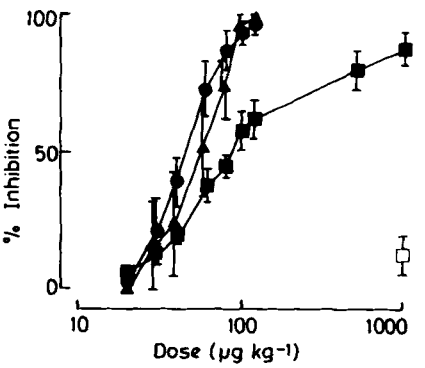

FIG. 2. Log dose-inhibition lines for the effects of HS-626 on responses of the anaesthetized cat tibialis anterior muscle (▲), of the soleus muscle (●), and of the heart rate to vagal stimulation (■). The effects of the highest concentration used are also shown on the response of the nictitating membrane to preganglionic stimulation (□). Standard errors are shown except where smaller than the symbols.

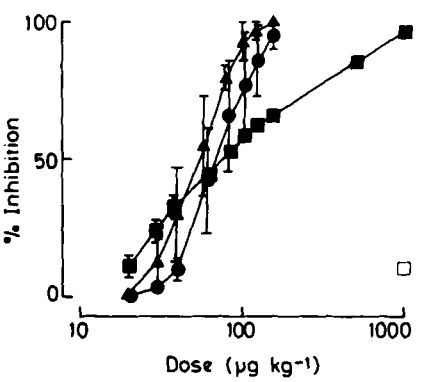

FIG. 3. Log dose-inhibition lines for the effects of HS-627 in the anaesthetized cat. Legend as for Figure 2.

Table 1. Doses of azasteroids producing 50% reduction of tibalis anterior and soleus muscle twitches, of bradycardia to vagal stimulation and of nictitating membrane responses to preganglionic stimulation in the anaesthetized cat. Values for chandonium, included for comparison, are from Teerapong et al (1979). Values are means ± s.e.m.

Compound	Tibialis anterior $\mu g\ kg^{-1}$	Soleus $\mu g\ kg^{-1}$	Vagus $\mu g\ kg^{-1}$	Nictitating membrane $\mu g\ kg^{-1}$	Vagus/soleus ratio
HS-626	57 ± 11	46 ± 6	89 ± 14	>1000	1·94 ± 0·14
HS-627	58 ± 11	74 ± 16	68 ± 4	>1000	1·02 ± 0·15
HS-408	>4000	>4000	410 ± 59	>4000	<0·1
HS-465	>4000	>4000	188 ± 54	1913 ± 711	<0·05
HS-522	>4000	>4000	750 ± 311	4150* ± 150	<0·19
HS-523	>4000	>4000	237 ± 114	5167* ± 833	<0·06
Chandonium	39 ± 11	47 ± 12	104 ± 22	3400 ± 1500	2·2

* by extrapolation.

ganglion block are in Table 1. Both HS-626 and HS-627 produced neuromuscular blockades of intermediate onset and duration (Table 2). Thus, the onsets of action of the compounds were not as quick as that of the steroidal neuromuscular blocker Org 6368 which has been tested under exactly the same conditions (Durant et al 1979). However, the recovery times were quicker than that of pancuronium (Durant et al 1979). The time course of action of the compounds in the cat is similar to that seen with the new steroidal relaxant Org NC45 (Durant et al 1979).

When the four compounds were compared with chandonium in the same cats the order of neuromuscular blocking potencies was HS-627 (2·0) > HS-626 (1·56) > chandonium (1·0) > HS-408 (0·0085) > HS-465 (0·004). In these experiments both HS-626 and HS-627 exhibited slightly less propensity than chandonium to block the responses of the heart rate to vagal stimulation. In contrast both HS-408 and HS-465 reduced the bradycardial responses to vagal stimulation and responses of the nictitating membrane at sub-neuromuscular blocking doses. A comparison of the effects of HS-626 and HS-465 is shown in Fig. 6.

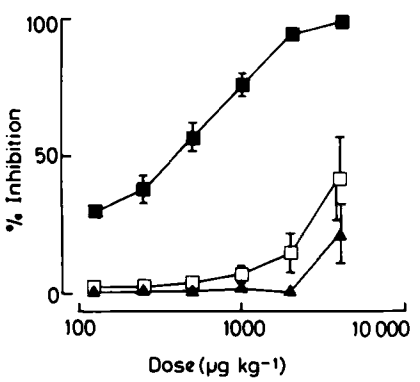

FIG. 4. Log dose-inhibition lines for the effects of HS-408 on responses of the heart rate of the anaesthetized cat to vagal stimulation (■), of the nictitating membrane to preganglionic stimulation (□), and of the tibialis anterior muscle (▲). Standard errors are shown except where smaller than the symbols.

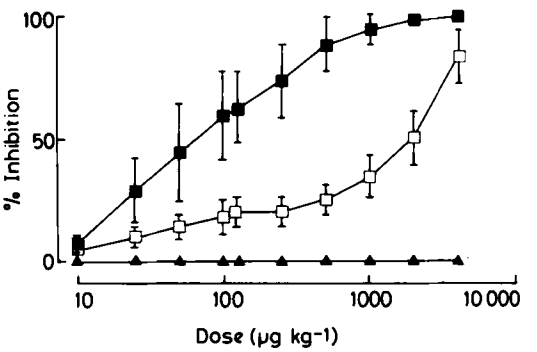

FIG. 5. Log dose-inhibition lines for the effects of HS-465 in the anaesthetized cat. Legend as for Fig. 4.

4-Aza-androstanes (HS-522 and HS-523) and 4-Aza-cholestanes (HS-405, HS-460 and HS-464). Neither HS-522 nor HS-523 exhibited marked neuromuscular blocking activity in doses up to 4 mg kg^{-1}. At 4 mg kg^{-1} HS-522 produced slight twitch augmentation in the tibialis anterior muscle of one cat and HS-523 produced slight twitch depression in the tibialis anterior of one cat. Both compounds produced 50% block of the bradycardial responses to vagal stimulation at doses less than 1 mg kg^{-1} and doses of the order of 4–6 mg kg^{-1} were required to block responses of the nictitating membrane to preganglionic stimulation (Table 1).

HS-405, HS-460 and HS-464 produced very small and inconsistent effects on neuromuscular, vagal and ganglionic transmission. At the highest dose used

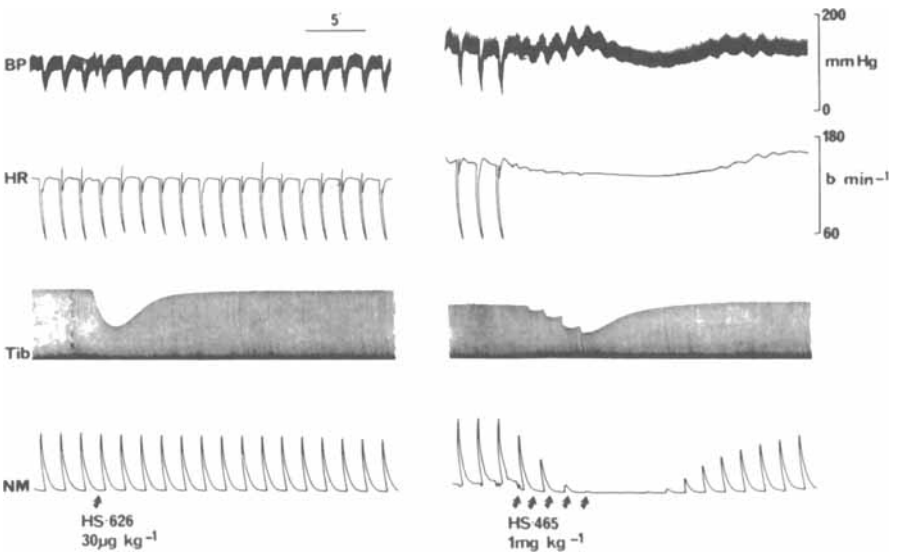

FIG. 6. Effects of HS-626 and HS-465 in the chloralose anaesthetized cat. Effects are shown on the blood pressure (BP), heart rate (HR) and responses of the heart rate to vagal stimulation (downward deflections), the tibialis anterior muscle (tib), and the preganglionically stimulated nictitating membrane (NM). Note that the bis-quaternary compound HS-626 produces neuromuscular block at a low dose. At this dose there is a slight reduction of the bradycardia produced by vagal stimulation, but no effect on the nictitating membrane. In contrast, the mono-quaternary compound HS-465 produces vagal block at lower doses than those reducing the responses of the nictitating membrane and of the tibialis anterior muscle.

Table 2. Time courses of 80–99% neuromuscular block produced by HS-626 and HS-627 in the anaesthetized cat. Values for chandonium, included for comparison, are from Teerapong et al (1979). Values are means ± s.e.m.

Compound	Muscle	Onset (min)	Duration to 95% recovery (min)	Recovery time (25–75% control) (min)
HS-626	Tibialis anterior	2·7 ± 0·1	11·0 ± 1·0	3·5 ± 0·1
HS-626	Soleus	3·0 ± 0·1	12·5 ± 0·7	3·4 ± 0·4
HS-627	Tibialis anterior	3·6 ± 0·3	11·9 ± 1·7	2·7 ± 0·5
HS-627	Soleus	4·7 ± 0·6	15·7 ± 2·5	4·3 ± 0·9
Chandonium	Tibialis anterior	2·1 ± 0·2	12·3 ± 2·5	3·2 ± 0·8
Chandonium	Soleus	3·9 ± 0·2	17·7 ± 0·9	3·8 ± 0·3

(4 mg kg^{-1}) these compounds slightly enhanced the responses of the tibialis anterior and soleus muscles. Similarly, bradycardial responses to vagal stimulation were usually enhanced. Conversely, responses of the nictitating membrane to preganglionic stimulation were usually depressed. All these drugs (HS-405, HS-460 and HS-464) were dissolved in a 50% ethanol solution, and equivalent amounts of this solution produced almost identical responses to those seen in the presence of the compounds.

Experiments on isolated preparations

Chick biventer cervicis preparation. Compounds HS-626 (0·32) and HS-627 (0·76) were slightly less potent than chandonium (1·0) in depressing the twitches of the stimulated chick biventer cervicis muscle preparation. The remaining compounds were approximately one-thousand times less active than chandonium. All compounds at concentrations that reduced twitch height also reduced responses to added acetylcholine.

Subsequent experiments on isolated preparations were confined to a comparison of the two most potent compounds HS-626 and HS-627 with chandonium. The pA_2 values for HS-626, HS-627 and chandonium against carbachol on the chick biventer cervicis muscle are shown in Table 3. The order of potency was chandonium (1·0) > HS-626 (0·27) > HS-627 (0·15).

Guinea-pig ileum preparation. HS-626, HS-627 and chandonium were approximately 100–300 times less potent in blocking responses of the guinea-pig ileum to acetylcholine than in blocking responses of the chick biventer cervicis muscle to carbachol. The order of potency of the compounds was chandonium (1·0) > HS-627 (0·55) > HS-626 (0·24) (Table 3).

Table 3. Comparison of the pA_2 values of HS-626, HS-627 and chandonium on nicotinic (chick biventer cervicis muscle), cardiac muscarinic (guinea-pig atria) and intestinal muscarinic (guinea-pig ileum) acetylcholine receptors. Values are means ± standard error of the mean (number of experiments is given in brackets).

Compound	Chick biventer (vs carbachol)	Guinea-pig atria (vs acetylcholine)	Guinea-pig ileum (vs acetylcholine)
Chandonium	8·6 ± 0·1 (4)	8·0 ± 0·2 (4)	6·1 ± 0·1 (4)
HS-626	8·1 ± 0·1 (7)	6·7 ± 0·2 (5)	5·5 ± 0·3 (8)
HS-627	7·8 ± 0·1 (7)	7·2 ± 0·1 (4)	5·9 ± 0·1 (8)

Guinea-pig atria preparations. HS-626, HS-627 and chandonium produced concentration-dependent rightwards shifts of the dose-response curve for the negative inotropic effects of acetylcholine on the electrically driven guinea-pig left atrium preparation. The three compounds were 16–80 times more potent in blocking acetylcholine responses in the atria than in the ileum (Table 3). The order of potency was chandonium (1·0) > HS-627 (0·16) > HS-626 (0·05).

HS-626, HS-627 and chandonium had no effect on the dose-response curve to the positive inotropic effects of noradrenaline on the electrically driven guinea-pig left atrium preparation. Occasionally the three compounds themselves produced positive inotropic effects.

Rat vas deferens preparation. HS-626, HS-627 and chandonium (10^{-4} M) increased responses to both nerve stimulation and to added noradrenaline on the rat isolated vas deferens preparation. Both types of response were also augmented by cocaine (10^{-6} M). There were no significant differences in the abilities of the compounds to augment responses to nerve stimulation, but chandonium was more effective than HS-626 and HS-627 in augmenting responses to added noradrenaline (Table 4).

DISCUSSION

The high neuromuscular blocking potency of pancuronium has been ascribed to the acetylcholine-like moieties in the molecule, especially that in the D ring (Buckett et al 1973; Durant et al 1979). The present compounds, and particularly HS-626 and HS-627, were synthesized with the aim of increasing the neuromuscular blocking potency without increasing the autonomic side effects of the compound by the incorporation of choline or acetylcholine-like moieties in the D ring of chandonium. Rather surprisingly, the choline analogue of chandonium, HS-626, proved to be equipotent as a neuromuscular blocking agent with HS-627, the acetylcholine analogue. In the pancuronium series, the 17-hydroxy analogue, dacuronium, is many times less potent than the 17-acetoxy compound, pancuronium (Durant et al 1979).

Table 4. Comparison of the augmenting effects of HS-626, HS-627 and chandonium on responses to nerve stimulation and to added noradrenaline in the rat isolated vas deferens.

Compound	Concn M	Percent. increase (± s.e.m.) Nerve stimulation	Noradrenaline
Chandonium	10^{-4}	30·6 ± 13·8	112 ± 26·2
HS-626	10^{-4}	32·8 ± 9·2	46 ± 10·8
HS-627	10^{-4}	65·5 ± 39	49·5 ± 16·6

When equiactive neuromuscular blocking doses of HS-626, HS-627 and chandonium were compared in the same cat, HS-626 possessed slightly less propensity than the other two compounds to block the bradycardial response to vagal stimulation. Comparison of the pA_2 values of the three bisquaternary compounds on the nicotinic receptors of the chick biventer cervicis muscle preparation and on the cardiac muscarinic receptors of the guinea-pig left atrium confirms that HS-626 had slightly more selectivity than HS-627 or chandonium for blocking the neuromuscular junction than for blocking the cardiac vagus neuroeffector junction. However, the vagus/soleus blocking ratio (Table 1) which is an indication of the margin between neuromuscular and vagal blocking activities in the cat is only around 2 for HS-626. This value is of the same order as those reported for alcuronium and pancuronium in the cat (Hughes & Chapple 1976; Durant et al 1979). Both alcuronium and pancuronium produce tachycardia in man (Kennedy & Kelman 1970; Kelman & Kennedy 1971) and therefore it is likely that HS-626 would also produce tachycardia due to vagal block.

None of the three bisquaternary compounds tested augmented cardiac responses to noradrenaline at concentrations at least ten times greater than those that produced anti-muscarinic effects. Nevertheless, all these compounds occasionally produced sympathomimetic effects on the isolated atria. Very high concentrations augmented responses of the vas deferens to nerve stimulation and to added noradrenaline which probably indicates a weak inhibitory action on neuronal noradrenaline uptake. Such an action has been postulated previously for chandonium (Harvey et al 1976; Marshall & Ojewole 1979). Thus, the inhibitory action of the compounds on cardiac muscarinic receptors is the predominant

side effect, but weak augmenting effects on sympathetic transmission to the heart may contribute to any tachycardia seen with the compounds.

All three bisquaternary compounds were about 100 times less potent in blocking the intestinal muscarinic receptors than in blocking cardiac muscarinic receptors. This is in keeping with results obtained for gallamine (Rathbun & Hamilton 1970) and pancuronium (Saxena & Bonta 1970; Marshall & Ojewole 1979) and is additional evidence that cardiac muscarinic receptors differ from those in the intestine.

The observations on the present series of compounds support previous findings that two nitrogens are essential for high neuromuscular blocking potency in the chandonium series (Gandiha et al 1974). All the monoquaternary compounds tested had autonomic blocking actions at doses lower than those producing neuromuscular block. None of the monoquaternary compounds tested in either this or previous studies possessed sufficient neuromuscular blocking potency to allow predictions about the relative importance of the ring A or ring D nitrogens in determining potency. In the present series of compounds the apparent activities of the two monoquaternary compounds HS-405, HS-464 and the tertiary compound HS-460 could be attributed to effects of the solvent system used.

In conclusion, although the 17a-(2-hydroxyethyl) analogue of chandonium (HS-626) possessed a slightly more desirable spectrum of neuromuscular and autonomic blocking activities than chandonium, the degree of improvement over the parent compound is insufficient to merit extensive clinical testing.

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