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The effects of dihydrochandonium and other chandonium analogues on neuromuscular and autonomic transmission

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The muscle relaxant chandonium and four of its analogues have been assessed for neuromuscular, ganglion blocking and vagolytic activity in the chloralose-anaesthetized cat, for neuromuscular blocking activity in the chick isolated biventer cervicis and rat phrenic nerve-hemidiaphragm preparations, for muscarinic and histamine blocking activity in the guinea-pig isolated ileum preparation and for effects on adrenergic responses in the rat isolated vas deferens preparation. All five compounds produced neuromuscular block of rapid onset and short duration when given to cats in doses of 5–500 μg kg^{-1} and produced non-depolarizing competitive neuromuscular blockade in the isolated skeletal muscle preparations. Saturation of the 5–6 androstene bond to produce dihydrochandonium (HS692) resulted in a loss of potency by a factor of 0·5. The *NN*-diethyl analogue of dihydrochandonium (HS693) was about half as potent as HS692. The triethyl (HS704) and *NN*-diethyl (HS705) analogues of chandonium possessed one-tenth and one-fifth respectively of the muscle relaxant activity of chandonium. In cats, all compounds inhibited the bradycardia produced by vagal stimulation and by methacholine, the vagolytic effect of HS693 being particularly powerful and long-lasting. Although the compounds did not block the depressor response to methacholine in vivo, all the compounds inhibited responses to carbachol in the guinea-pig isolated ileum. However, from pA_2 values the compounds were found to be 10–100 times more active at nicotinic than at muscarinic receptors. None of the compounds had histamine-blocking activity, ganglion-blocking activity, or actions on adrenergic responses.

Several bisquaternary azasteroid compounds have been shown to exhibit neuromuscular blocking activity (Marshall et al 1973a,b; Gandiha et al 1974, 1975). The most active compound, chandonium iodide, (17a-methyl-3β-pyrolidino-17a-aza-D-homo-5-androstene dimethiodide, HS310) has a neuromuscular blocking potency in the cat of around one-half of that of pancuronium, the most potent muscle relaxant in clinical use (Gandiha et al 1975). Chandonium has negligible ganglion blocking activity but exhibits vagolytic activity at neuromuscular blocking doses (Gandiha et al 1975). This vagolytic activity is likely to be associated with the production of tachycardia in anaesthetized man.

Four new close analogues of chandonium (Fig. 1) have now been synthesized (Singh et al 1979) in an attempt to gain insight into the structural requirements for neuromuscular blocking potency and for the undesirable autonomic blocking actions in chandonium-like compounds. The effects of the new compounds have been compared with those of chandonium in the anaesthetized cat, on isolated smooth and skeletal muscle preparations and on cholinesterase.

Some of these result have been presented to the British Pharmaceutical Conference, Sheffield, 1977 (Teerapong et al 1977).

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METHODS

Anaesthetized cat preparations

Mongrel cats of either sex (2–4 kg) were anaesthetized with a mixture of α-chloralose (80 mg kg^{-1}) and pentobarbitone sodium (50 mg kg^{-1}). Animals were artificially ventilated (18 ml air kg^{-1}; 26 breaths min^{-1}) throughout the experiments. Arterial blood pressure was recorded from a polythene cannula inserted in a femoral artery and connected to a Statham P23Ac pressure transducer. The blood pressure pulse was also used to trigger a cardiotachometer (Grass 7P4F) to record heart rate. Drugs were injected through a polythene cannula inserted in a femoral vein. The right vagus was ligated and stimulated on the peripheral side of the ligature every 100 s with 10 s trains of rectangular pulses (0·5 ms duration, 10 Hz freq.). The stimulation strength was adjusted to produce a decrease in heart

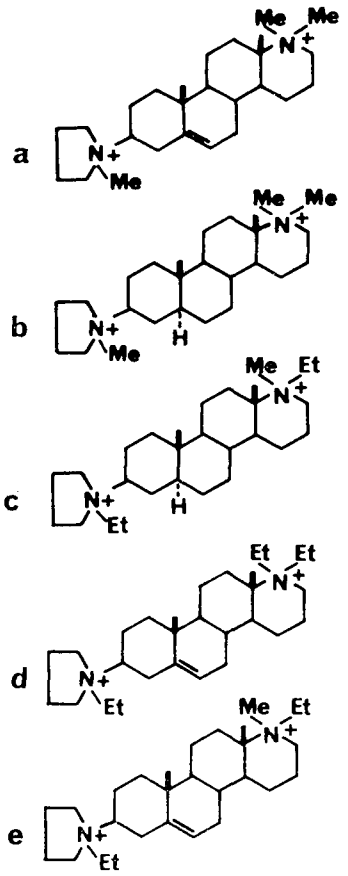

FIG. 1. Structures of chandonium (a) and its four analogues, HS-692 (b), HS-693 (c), HS-704 (d) and HS-705 (e).

rate of approximately 50%. Both cervical nerves were ligated and the left nerve was stimulated preganglionically with trains of pulses as used for vagal stimulation. In some experiments the right cervical nerve was ligated and stimulated postganglionically. Contractions of the nictitating membranes were recorded by Grass FTO3C force displacement transducers. The left sciatic nerve was ligated and crushed in the popliteal space. The 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. In some experiments the tibialis anterior muscle was stimulated directly using 1 ms duration pulses applied between a needle electrode inserted in the belly of the muscle and a metal pin inserted through the knee joint. In these experiments neuromuscular transmission was blocked by tubocurarine (1 mg kg^{-1}). In some experiments in cats treated with atropine (1 mg kg^{-1}), nerve stimulation was temporarily stopped and acetylcholine was injected close-arterially into the tibialis anterior muscle by the method of Brown (1938) as modified by Blaber (1960).

Only one compound was tested in each cat, except in experiments using directly elicited contractions, postganglionic stimulation of the nictitating membrane or close-arterial administration of acetylcholine. Test drugs were administered at intervals of at least 1 h depending on the depth of blockade produced.

Chick biventer cervicis muscle preparation

The chick biventer cervicis nerve-muscle preparation (Ginsborg & Warriner 1960) was mounted in Krebs-Henseleit (1932) solution at 32 °C and bubbled with oxygen containing 5% carbon dioxide. The nerve within the 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 carbachol was added and allowed to remain in the tissue bath for 60 s before washout by overflow for 30 s. Dose-response curves to carbachol were constructed by increasing successive doses until maximum contractures were produced. Dose-response curves to carbachol were constructed in several concentrations of each chandonium analogue and the pA_2 value of the antagonist was calculated from extrapolation of the plot of log (dose ratio-1) against pA_x (Arunlakshana & Schild 1959). In some experiments neuromuscular transmission was abolished by (+)-tubocurarine (10^{-5} M) and the muscle was stimulated directly with pulses of 1 ms duration and strength sufficient to produce maximal twitches.

Rat phrenic nerve-hemidiaphragm preparation

The rat phrenic nerve-hemidiaphragm preparation (Bülbring 1946) was mounted and stimulated under identical conditions to those described for the chick biventer cervicis muscle.

Guinea-pig ileum preparation

Segments of guinea-pig terminal ileum were mounted in Tyrode (1910) solution at 32°C and bubbled with air. Dose-response curves to carbachol were constructed by increasing successive doses until maximal responses were obtained. Dose-response curves were constructed in the presence of several concentrations

of the chandonium analogues and the pA_2 values of the compounds were obtained as described for the chick biventer cervicis muscle preparation. Responses were measured isometrically by UFI strain gauges.

Rat vas deferens preparation

Rat vasa deferentia were mounted in Krebs-Henseleit solution containing atropine (2×10^{-6} M) and hexamethonium (6×10^{-5} M) at 32 °C and bubbled with oxygen containing 5% carbon dioxide. The preparations were stimulated every 20 min with 10 s trains of impulses (0·5 ms duration, 20 Hz frequency) applied through ring electrodes placed around the mid-portion of the vas deferens. Periodically noradrenaline (3×10^{-5}M) was added to the tissue for 30 s.

Cholinesterase determinations

The anticholinesterase activities of the chandonium analogues were assessed by measuring the cholinesterase activity of intact chick biventer cervicis muscles and homogenates of cat tibialis anterior muscles in the presence of the drugs, using the colorimetric method of Ellman et al (1961). Homogenates were prepared using 1 g of tissue in 28·6 ml of 0·1 M phosphate buffer (pH 8·0) and a final concentration of 10 mg homogenate/ml reaction mixture was used for each assay. Three biventer cervicis muscles from 7–14 day old chicks were used for each assay on intact tissue. The reaction flask contained 5·8 ml of 0·1M phosphate buffer (pH 8·0) and was maintained at 37 °C. The reaction was begun by addition of 0·2 ml acetylthiocholine iodide (0·075 M). At 15 min intervals for 1 h, 0·2 ml was removed and added to a cuvette containing 2·9 ml 0·1 M phosphate buffer (pH 8·0) and 0·1 ml 5,5-dithiobis-2-nitrobenzoic acid (0·01M). Blank determinations were performed on flasks containing tissue but no substrate and on flasks with all the reagents but no tissue. The absorbance change min^{-1} at 412 nm in control preparations was compared with that in the presence of the test compounds. Concentrations of the chandonium analogues producing 50% inhibition of the enzyme were calculated.

Drugs

The drugs used were: acetylcholine chloride, acetyl-β-methylcholine chloride, acetylthiocholine iodide, 4-aminopyridine, atropine sulphate, carbachol chloride, α-choralose, 5,5-dithiobis-2-nitrobenzoic acid, noradrenaline hydrochloride, succinylcholine chloride, (+)-tubocurarine chloride (Sigma); edrophonium chloride, neostigmine methylsulphate (Roche); 3,4-diaminopyridine, hexamethonium bromide (Koch-Light); histamine acid phosphate (British Drug Houses); pentobarbitone sodium (Abbott); pancuronium bromide (Organon); dimethylphenylpiperazinium iodide (synthesized by Dr A. L. Green, Department of Biochemistry, University of Strathclyde); methoxamine hydrochloride (Wellcome); mepyramine maleate (May and Baker); cocaine hydrochloride (MacFarlan Smith).

Treatment of results

Statistical evaluation was performed using unpaired Student's *t*-test or the Mann-Whitney U-test. Differences between the means were taken as significant when $P < 0.05$.

RESULTS

Anaesthetized cats

Neuromuscular blocking action

All four compounds inhibited the responses of the tibialis anterior and soleus muscles to stimulation of the sciatic nerve. The effective dose range was from 20–500 $\mu g\ kg^{-1}$ whereas doses up to 1 mg kg^{-1} were without effect on directly elicited twitches. The neuromuscular blockade was not preceded by augmentation of the muscle twitches or accompanied by fasciculation or contracture. The compounds showed a slightly greater blocking action on the fast contracting tibialis anterior than on the slow-contracting soleus muscle (Table 1 and Fig. 2) and twitches of the tibialis anterior muscle recovered faster from the block than twitches of the soleus muscle. HS692 was the most potent of the four analogues (Fig. 3) although only half as potent as the parent compound chandonium. The order of potency relative to chandonium as 1·0 was: HS692 (0·52) >HS693 (0·22) ≃HS705 (0·19) >HS704 (0·11). Except for the difference in potency between compounds HS693 and HS705 all the differences between compounds were statistically significant ($P < 0.05$).

The four compounds produced neuromuscular block of rapid onset and short duration that approached that produced by suxamethonium (Table 2 and Fig. 4). There was no significant difference in onset, duration or recovery time between the four compounds and chandonium, although they were faster in onset and shorter acting than pancuronium and (+)-tubocurarine (Fig. 4).

The neuromuscular blockade produced by the four analogues, like that produced by chandonium, was rapidly reversed by injection of the anticholinesterases neostigmine (100 $\mu g\ kg^{-1}$) or edrophonium

Table 1. Neuromuscular, vagal and ganglion blocking potencies of chandonium and its analogues in the chloralose-anaesthetized cat. ED50 values are doses required to produce 50% inhibition of responses of tibialis anterior and soleus muscles to sciatic nerve stimulation, and of the bradycardia in response to vagal stimulation. ED50 values are also related to the appropriate value for chandonium. (Rel: chand.) Values represent mean ± s.e.m. of observations in 4–12 separate cats.

	Neuromuscular blocking activity				Vagal blocking activity		ED50 vagus:	Ganglion blocking activity
	Tibialis		Soleus					
Compound	ED50 (μg kg^{-1})	Rel: chand	ED50 (μg kg^{-1})	Rel: chand	ED50 (μg kg^{-1})	Rel: chand	ED50 tibialis	(% inhibition at 1 mg kg^{-1})
Chandonium	39 ± 11	1·00	47 ± 12	1·00	104 ± 22	1·00	2·7	3·4 ± 1·5
HS692	80 ± 10	0·49	86 ± 12	0·55	58 ± 8	1·79	0·7	6·8 ± 3·0
HS693	190 ± 16	0·21	213 ± 18	0·22	18 ± 5	5·78	0·1	4·7 ± 1·1
HS704	336 ± 80	0·12	465 ± 96	0·10	52 ± 12	2·00	0·2	6·5 ± 1·3
HS705	218 ± 25	0·18	222 ± 17	0·21	95 ± 3	1·09	0·4	6·7 ± 2·4

(1 mg kg^{-1}). The effects of the chandonium analogues were also reversed by administration of 4-aminopyridine or 3,4-diaminopyridine (1 mg kg^{-1}), agents that increase the release of acetylcholine in response to nerve stimulation (Harvey & Marshall 1977 a, b). All the compounds, in doses that abolished contractions to indirect stimulation, abolished the response of the tibialis anterior muscle to close-arterial injection of acetylcholine. The response to exogenous acetylcholine recovered as the twitch blockade waned.

Vagolytic and ganglion blocking actions

Like chandonium, the compounds reduced the bradycardial response to vagal stimulation. With HS692 the doses producing vagolytic effects were similar to those producing muscle paralysis (Figs 3 and 5) but with HS693, HS704 and HS705 the vagolytic action was seen at sub-neuromuscular blocking doses, although the maximal blockade of vagal stimulation occurred at doses similar to those giving complete twitch blockade. HS693 had the most powerful vagolytic action, being 9 times more potent than chandonium, and was particularly prolonged in effect (Fig. 6). For example, a dose of 100 μg kg^{-1} HS693 produced about 40% depression of twitches with complete recovery in 10 min whereas the responses to vagal stimulation were depressed by 90% and recovered to 90% of control around 80 min later. All four analogues inhibited the bradycardia but not the depressor effect of acetyl-β-methylchol-

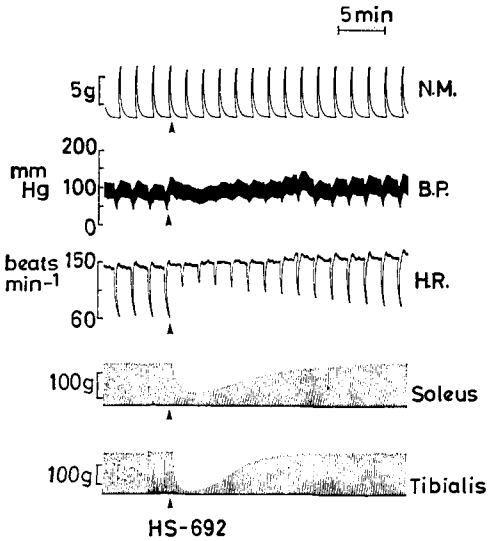

FIG. 2. Chloralose-anaesthetized cat: The effects of a neuromuscular blocking dose of HS692 (250 μg kg^{-1}) on the preganglionically stimulated nictitating membrane (N.M.) (10 Hz for 10 s), arterial blood pressure (B.P.), heart rate (H.R.) including bradycardial responses to vagal stimulation (10 Hz for 10 s), and the soleus and tibialis anterior muscles (0·1 Hz).

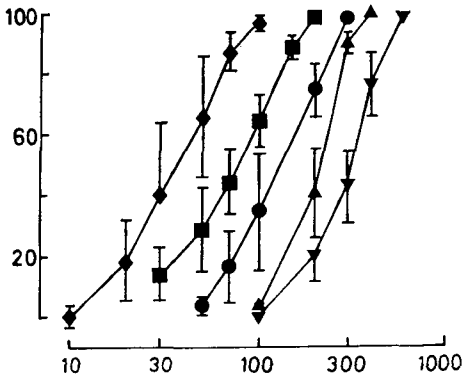

FIG. 3. Comparison of the neuromuscular blocking potencies of chandonium (◆), HS692 (■), HS693 (●), HS704 (▼) and HS705 (▲) on the responses of the tibialis anterior muscle of the cat to single shock nerve stimulation (0·1 Hz). Each point represents the mean (± s.e.m.) of at least five determinations. Vertical lines indicate standard errors (except where smaller than the symbol). Ordinate: percentage inhibition. Abscissa: dose (μg kg^{-1}).

Table 2. Onset, duration and recovery time of neuromuscular blockade at 90–99% inhibition produced by chandonium iodide and its four analogues in the chloralose-anaesthetized cat. Onset is time from injection to maximum effect. Duration is time from maximum effect to 90% recovery. Recovery time is time from 25–75% recovery. Values represent the mean ± s.e.m. of four determinations, except for HS705 where the values are from one determination.

Compounds (μg kg^{-1})	Muscle	Onset (min)	Duration (min)	Recovery time (min)
Chandonium	Tibialis	2·1 ± 0·2	12·3 ± 2·5	3·2 ± 0·8
(50–150)	Soleus	3·9 ± 0·2	17·7 ± 0·9	3·8 ± 0·3
HS692	Tibialis	2·4 ± 0·1	14·5 ± 1·8	4·6 ± 0·8
(100–200)	Soleus	3·2 ± 0·5	17·2 ± 3·9	5·2 ± 1·3
HS693	Tibialis	2·6 ± 0·1	13·5 ± 1·6	4·3 ± 0·5
(200–300)	Soleus	3·8 ± 0·4	23·9 ± 1·7	6·7 ± 0·6
HS704	Tibialis	2·1 ± 0·2	12·4 ± 0·5	3·9 ± 0·4
(400–600)	Soleus	3·6 ± 0·3	22·9 ± 5·2	6·6 ± 1·6
HS705	Tibialis	2·5	14·8	3·6
(300)	Soleus	3·6	25·8	6·8

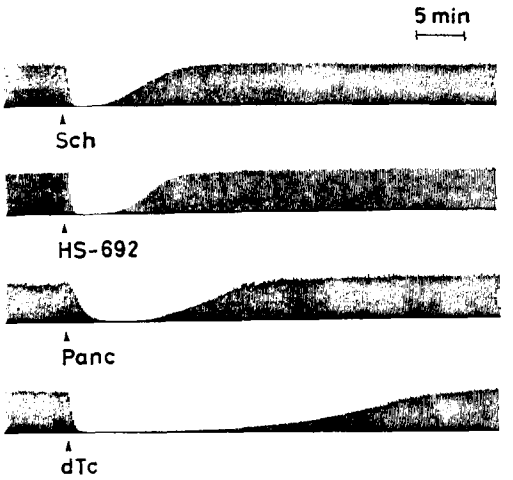

FIG. 4. Chloralose-anaesthetized cat: Comparison of the durations of action of suxamethonium (SCh) 85 μg kg^{-1}, HS692, 250 μg kg^{-1}, pancuronium (Panc) 15 μg kg^{-1} and tubocurarine (dTc) 250 μg kg^{-1} on the responses of the tibialis anterior muscle to single shock nerve stimulation.

ine (10–15 μg kg^{-1}) indicating that the compounds have an atropine-like effect at the cardiac vagus neuroeffector junction but not a generalized atropine-like action.

The compounds possessed little ganglion blocking activity as assessed by reduction in the response of the nictitating membrane to preganglionic stimulation. Doses up to 1 mg kg^{-1}, i.e. above the level required for complete block of neuromuscular transmission, produced only a slight inhibition of the contractions of the nictitating membrane (Table 1). Responses of the nictitating membrane to postganglionic stimulation of the cervical sympathetic nerve were not depressed in the presence of any of

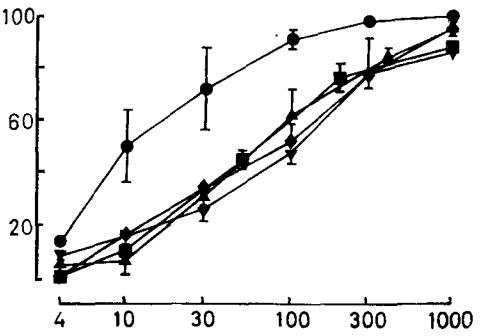

FIG. 5. Comparison of inhibitory effects of chandonium (◆), HS692 (■), HS693 (●), HS704 (▲) and HS705 (▼) on the responses of the heart rate of the cat to vagal stimulation (10 Hz for 10 s). Each point represents the mean (± s.e.m.) of at least five determinations. Vertical lines indicate standard errors (except where smaller than the symbol). Ordinate: percentage inhibition. Abscissa: dose (μg kg^{-1}).

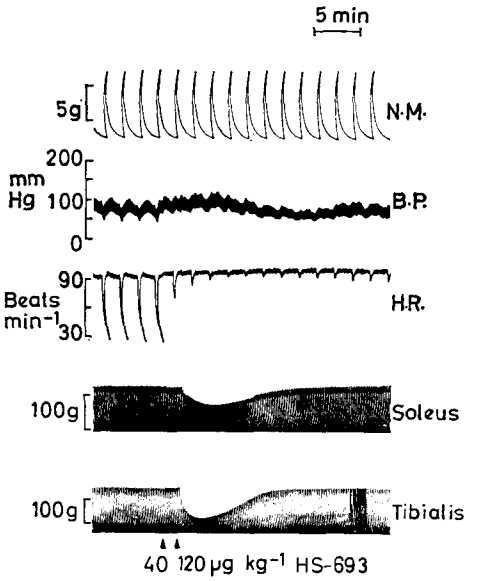

FIG. 6. Chloralose-anaesthetized cat: The effects of a neuromuscular blocking dose (120 μg kg^{-1}) of HS693 on the preganglionically stimulated nictitating membrane (N.M.), arterial blood pressure (B.P.), heart rate (H.R.), and the soleus and tibialis anterior muscles. Note that the vagolytic effect of HS693 is particularly long lasting.

the compounds. In fact, the compounds at a concentration of 3 mg kg^{-1} increased the contractions of the nictitating membrane and there was an increase in resting tension.

In cats treated with atropine (1 mg kg^{-1}) the pressor effects of the ganglion stimulant dimethylphenylpiperazinium (10–20 μg kg^{-1}) and of nor-

adrenaline (5–15 μg kg^{-1}) were unaffected by neuromuscular blocking doses of the four compounds.

Isolated preparations

Chick biventer cervicis muscle preparation

All four analogues depressed the indirectly but not the directly elicited twitches without causing any contracture of the muscle. The effects of the compounds were readily reversed by washing the preparation, whereas recovery from tubocurarine-induced blockade was much slower. The neuromuscular blockade produced by the compounds was rapidly reversed by neostigmine (3×10^{-6} M, 1·0 μg ml^{-1}) and by 4-aminopyridine (10^{-4} M, 10 μg ml^{-1}).

The compounds were found to inhibit the responses to added acetylcholine and carbachol and produced a progressive, concentration-dependent parallel shift to the right of the concentration-effect curve to carbachol. Plots of log (dose ratio-1) against the negative log of the molar concentration of antagonist (pA_x) produced straight lines with slopes not significantly different from unity (Table 3). All compounds were at least ten times more active than (+)-tubocurarine, and their potency relative to chandonium was similar to that found in the anaesthetized cat: HS692 (0·4) > HS693 (0·2) > HS705 (0·1) > HS704 (0·07) > (+)-tubocuraine (0·009).

Rat phrenic nerve-hemidiaphragm preparation

As in the chick muscle preparation, the compounds all reduced the twitch responses of the rat hemidiaphragm to indirect stimulation. Again, the muscle paralysis caused by the chandonium analogues was more rapidly reversed by washing than was that caused by tubocurarine, and the effects of the chandonium analogues were readily reversed by neostigmine or 4-aminopyridine. The reversal by neostigmine was generally only to about 50% of control height but 4-aminopyridine often returned the twitch response to a level above that of control.

The ED50 values for tubocurarine, chandonium and the four analogues are shown in Table 3. As might be expected (Bowman 1964) tubocurarine is much more effective in blocking the rat muscle preparation. The difference in potencies between chandonium and its analogues is much less than in the cat or the chick preparations, and the order of potency is also changed: chandonium (1·0) =HS693 (1·0) > HS705 (0·6) = HS692 (0·6) > HS704 (0·5).

Table 3. pA_2 values of chandonium iodide and its four analogues on chick biventer cervicis and guinea-pig ileum preparations.

Antagonist	Chick biventer cervicis pA_2	Chick biventer cervicis slope	Guinea-pig ileum pA_2	Guinea-pig ileum slope	Rat diaphragm ED50(M)	Rat diaphragm Rel: chand
Chandonium	8·05	−0·96	5·8	−0·92	$2·7 \times 10^{-6}$	1·0
HS692	7·6	−1·11	5·5	−0·98	$4·7 \times 10^{-6}$	0·57
HS693	7·4	−1·22	6·2	−0·71	$2·8 \times 10^{-6}$	0·97
HS704	6·9	−1·04	6·1	−1·06	$5·5 \times 10^{-6}$	0·49
HS705	7·1	−0·99	5·7	−1·07	$4·4 \times 10^{-6}$	0·61
(+)-Tubocurarine	6·0*				$9·5 \times 10^{-7}$	28·1

* Value from Gandiha et al 1972.

Guinea-pig ileum preparation

In concentrations above $1·5 \times 10^{-6}$ M (1·0 μg ml^{-1}) all of the compounds produced concentration-dependent parallel shifts to the right of the dose-response curve to carbachol. The pA_2 values ranged from 5·5 for HS692 to 6·2 for HS693 and the slopes of the Arunlakshana & Schild (1959) plots were close to unity (Table 3). The order of potency relative to chandonium was HS693 (2·5) > HS704 (2·0) > chandonium (1·0) > HS705 (0·8) > HS692 (0·5).

When tested against contractions of the ileum produced by histamine (3×10^{-7} M) the compounds in concentrations up to 3×10^{-5} M had no significant effect whereas mepyramine (5×10^{-8} M) was found to abolish the standard histamine responses.

Rat vas deferens preparation

The compounds produced a concentration-dependent augmentation of the responses to exogenous noradrenaline (3×10^{-5} M; 10 μg ml^{-1}) but not to methoxamine (4×10^{-5} M; 10 μg ml^{-1}). The order of potency relative to chandonium was HS693 (1·7) >HS704 (1·3) > chandonium (1·0) > HS705 (0·7) >HS692 (0·6). However, from a determination of concentrations required to increase the noradrenaline response by 50% it was estimated that HS693 was about 50 times less potent than cocaine in this action. The second phase of the biphasic response to electrical stimulation can be augmented by cocaine (10^{-6} -10^{-5} M). The chandonium analogues also increased the size of the responses to electrical stimulation, although concentrations as high as 100 μg ml^{-1} (about 2×10^{-4} M) were necessary to reveal an effect. Additionally, the amount of augmentation was small (about 40%) compared to the 150% increase possible with cocaine.

Acetylcholinesterase activity

The anticholinesterase activities of test compounds

and chandonium were compared to that of neostigmine in homogenates of cat tibialis anterior muscle and intact chick biventer cervicis muscles. All of the compounds possessed very weak anticholinesterase activity. HS693, which was the most powerful enzyme inhibitor of the chandonium analogues, was over 1000 times less potent than neostigmine when tested on the cat tibialis muscle homogenate and about 140 times less potent than neostigmine on the intact chick biventer cervicis muscles (Table 4). HS704 showed barely detectable activity on both preparations at concentrations up to 5×10^{-5} M.

Table 4. Comparison of the concentrations producing 50% inhibition of cholinesterase activity.

	Concentrations (M) producing 50% ChE inhibition	
Compounds	Cat tibialis	Chick biventer
Chandonium	$1{\cdot}3 \times 10^{-4}$	$1{\cdot}4 \times 10^{-4}$
HS692	$1{\cdot}7 \times 10^{-4}$	$4{\cdot}8 \times 10^{-5}$
HS693	$1{\cdot}4 \times 10^{-4}$	$2{\cdot}7 \times 10^{-5}$
HS704	$2{\cdot}1 \times 10^{-5}$	$6{\cdot}8 \times 10^{-5}$
HS705	$8{\cdot}5 \times 10^{-4}$	$6{\cdot}5 \times 10^{-5}$
Neostigmine	$1{\cdot}2 \times 10^{-7}$	$1{\cdot}9 \times 10^{-7}$

DISCUSSION

All four of the analogues of chandonium iodide possessed neuromuscular blocking activity in the anaesthetized cat and in the isolated rat phrenic nerve-hemidiaphragm and chick biventer cervicis nerve-muscle preparations. The compounds did not produce contracture or fasciculation of skeletal muscle, and were readily reversed by anticholinesterases, indicating that they possess a non-depolarizing, tubocurarine-like action. As the Arunlakshana & Schild (1959) plots approximated to unity, it may be deduced that the antagonism is competitive in nature.

In vivo, the compounds had little ganglion blocking activity but, like chandonium, they could inhibit the negative chronotropic response to vagal stimulation. The antagonism at the cardiac vagus does not appear to be caused by general muscarinic receptor blocking activity as the depressor effects of the muscarinic stimulant acetyl-β-methylcholine were not inhibited by concentrations of the compounds that reduced its negative chronotropic effects. Also, although the compounds exhibited weak muscarinic antagonism in the guinea-pig ileum preparation, there was no exact correlation between blocking potencies on the ileum and at the cardiac vagus neuroeffector junction.

Recently Barlow et al (1976) have suggested that muscarinic receptors of atria and ileum are not identical. HS693 was the most active of the compounds tested as a vagal blocker and its action was particularly long lasting. Vagolytic effects have been used to explain the tachycardia produced by many muscle relaxants in man (Riker & Wescoe 1951; Saxena & Bonta 1970; Lee Son & Waud 1977) and, in fact, chandonium produces tachycardia in man (S. Agoston—personal communication). An adrenergic component has also been implicated in the tachycardia produced by gallamine and pancuronium (Brown & Crout 1970; Ivankovitch et al 1975; Domenech et al 1976; Docherty & McGrath 1978). These compounds have been shown to release noradrenaline or to inhibit neuronal uptake of noradrenaline. Chandonium has previously been shown to increase responses to added noradrenaline in the guinea-pig isolated vas deferens preparation (Harvey et al 1976). This action is probably indicative of a weak inhibitory effect on noradrenaline uptake. Such an action on the heart, along with the vagolytic effect, would tend to produce tachycardia. Thus, although the compounds produce non-depolarizing competitive neuromuscular blockade of rapid onset and short duration in the cat, they would not appear to offer any potential clinical advantage over the parent compound, chandonium, which is two to ten times more potent. However, the close structural resemblance between chandonium and the analogues may permit some conclusions to be made concerning the structural requirements for a muscle relaxant compound with no disadvantageous cardiovascular side effects.

Saturation of the double bond in the androstene nucleus of chandonium gives HS692, dihydrochandonium, which should be a more flexible molecule. The loss of blocking potency suggests that HS692 does not bind as well as chandonium to nicotinic receptors: perhaps the flexibility allows a deformation of the molecule to bind to subsites adjacent to the receptor.

Introduction of an ethyl group on to each quaternary nitrogen of both chandonium and dihydrochandonium leads to a significant diminution of neuromuscular blocking potency (HS705 and HS693 respectively). Interestingly, the loss of activity on diethyl substitution was much greater in chandonium than in dihydrochandonium, again indicating that small changes in structure profoundly affect activity in chandonium, and suggesting that chandonium fits the receptor very closely. Triethyl substitution in chandonium predictably caused a further significant

reduction of neuromuscular blocking activity. These results show that increased bulk of the substituents leads to decreased neuromuscular blocking potency, reflecting the need for the quaternary sites to resemble that of acetylcholine for efficient binding to occur.

Both increasing flexibility of the steroid nucleus and introducing ethyl substituents not only decreased neuromuscular blocking potency but increased the unwanted blocking activity at the cardiac vagus neuroeffector junction. This indicates that the receptors at these two sites are different and suggests that it may be possible to synthesize compounds in this series with selective blocking actions at the neuromuscular junction.

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REFERENCES

Arunlakshana, O., Schild, H. O. (1959) Br. J. Pharmacol. Chemother. 14: 48–58

Barlow, R. B., Berry, K. J., Glenton, P. A. M., Nikolaou, N. M., Son, K. S. (1976) Br. J. Pharmacol. 58: 613–620

Blaber, L. C. (1960) Br. J. Pharmacol Chemother. 15: 476–484

Bowman, W. C. (1964) in: Laurence, D. R., Bacharach, A. L. (eds) Evaluation of Drug Activities: Pharmacometrics. Academic Press, London, Vol. 2, pp. 325–351

Brown, G. L. (1938) J. Physiol. Lond. 92: 22–23P

Brown, B. R., Crout, J. R. (1970) J. Pharmacol. Exp. Ther. 172: 266–273

Bülbring, E. (1946) Br. J. Pharmacol. Chemother. 1: 38–61

Docherty, J. R., McGrath, J. C. (1978) Br. J. Pharmacol. 64: 589–599

Domenech, J. S., Garcia, R. C., Sasian, J. M. R., Loyola, A. Q., Oroz, J. S. (1976) Br. J. Anaesth. 48: 1143–1148

Ellman, G. L., Courtney, K. D., Andres, V., Featherstone, R. M. (1961) Biochem. Pharmacol. 7: 88–95

Gandiha, A., Green, A. L., Marshall, I. G. (1972) Eur. J. Pharmacol. 18: 174–182

Gandiha, A., Marshall, I. G., Paul, D., Singh, H. (1974) J. Pharm. Pharmacol. 26: 871–877

Gandiha, A., Marshall, I. G., Paul, D., Rodger, I. W., Scott, W., Singh, H. (1975) Clin. Exp. Pharmacol. Physiol. 2: 159–170

Ginsborg, B. L., Warriner, J. (1960) Br. J. Pharmacol. Chemother. 15: 410–411

Harvey, A. L., Marshall, I. G. (1977a) Eur. J. Pharmacol. 44: 303–309

Harvey, A. L., Marshall, I. G. (1977b) Naunyn-Schmiedeberg's Arch. Pharmacol., 299: 53–60

Harvey, A. L., Paul, D., Rodger, I. W., Singh, H. (1976) J. Pharm. Pharmacol. 28: 617–619

Krebs, H. A., Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. 210: 33–66

Ivankovitch, A. D., Miletich, D. J., Albrecht, R. F., Zahed, B. (1975) J. Pharm. Pharmacol., 27: 837–841

Lee Son, S., Waud, B. E. (1977) Anesthesiology 47: 34–36

Marshall, I. G., Paul, D., Singh, H. (1973a) J. Pharm. Pharmacol. 25: 441–446

Marshall, I. G., Paul, D., Singh, H. (1973b) Eur. J. Pharmacol. 22: 129–134

Riker, W. F., Wescoe, W. C. (1951) Ann. N.Y. Acad. Sci. 54: 373–392

Saxena, P. R., Bonta, I. L. (1970) Eur. J. Pharmacol. 11: 332–341

Singh, H., Bhardwaj, T. R., Ahuja, N. K., Paul, D. (1979) J. Chem. Soc. Perkin I, 305–307

Teerapong, P., Marshall, I. G., Harvey, A. L., Singh, H., Paul, D., Bhardwaj, T. R., Ahuja, N. K. (1977) J. Pharm. Pharmacol. 29 (Suppl.) 80P

Tyrode, M. V. (1910) Arch. Int. Pharmacodyn. Ther. 20: 205–223