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NEUROMUSCULAR BLOCKING ACTIVITIES OF SOME STEROIDAL MONO AND BIS-QUATERNARY AMMONIUM COMPOUNDS WITH SPECIAL REFERENCE TO *NN'*-DIMETHYLCONESSINE

BY

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The pharmacological properties of conessine, 3-β-dimethylamino-con-5-ene, a steroidal alkaloid obtained from the bark and seeds of *Holarrhena antidysenterica* have been described (Burn, 1914; Stephenson, 1948). Quaternary salts of the alkaloid have been prepared (Polstorff & Schirmer, 1886; Bertho, 1944) but do not seem to have been examined pharmacologically. We report here the results of an investigation of structure-activity relationships in a series of quaternary ammonium compounds derived from conessine or related steroids which possess neuromuscular blocking properties of the non-depolarizing type. The results for one compound, *NN'*-dimethylconessine, which is typical of this group and has been submitted for clinical trial, are recorded in detail.

METHODS

Cats

Adult cats of either sex were anaesthetized by intraperitoneal injection of chloralose (80 mg/kg) with pentobarbitone sodium (10 mg/kg). The right hind limb was set up in the horizontal position on a Brown-Schuster myography stand. The sciatic nerve was ligated, cut centrally and stimulated peripherally using shielded silver electrodes. Twitches and tetani were elicited in the tibialis anterior and soleus muscles by square wave pulses of 0.2 msec duration and of twice the strength required to produce maximal twitches of the muscles. The muscles were attached to flat steel springs and the contractions recorded semi-isometrically on a smoked paper. The muscles were warmed with a lamp and kept moist with liquid paraffin saturated with physiological saline. In some experiments, stimuli of supramaximal strength and 0.5 msec duration were applied directly to the tibialis anterior muscle between the tendon and the drill in the femur, using shielded silver wire. Close-arterial injections were made to the tibialis anterior muscle as described by Brown (1938). Gross muscle action-potentials were recorded on a Tektronix 502 dual-beam oscilloscope from platinum electrodes inserted through the belly and tendon of the tibialis muscle, and the antidromic nerve action potentials were recorded from shielded platinum electrodes placed on the sciatic nerve. Drugs were injected into the right external jugular vein through a tap cannula, and the left carotid arterial pressure was recorded using a mercury manometer. Artificial respiration was applied through a tracheal cannula connected to a Palmer respiration pump.

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In some experiments, in which the effects of volatile anaesthetics on muscle relaxant activity were being investigated, the cats were anaesthetized by intraperitoneal injection of thiopentone sodium (50 mg/kg) and maintained with ether or halothane administered on "open-circuit" using a Boyle's apparatus connected to a Palmer respiration pump.

Cardiovascular activity was investigated in chloralose-anaesthetized, artificially respired cats in which the carotid arterial blood pressure and electrocardiograph (lead II) were monitored. The responses of the nictitating membrane to supramaximal preganglionic stimulation of the cervical sympathetic nerve, and of the cardiovascular system to stimulation of the peripheral end of the cut vagus nerve were recorded. Drugs were injected into the saphenous vein through a tap cannula.

Monkeys

Cynomolgus monkeys of either sex weighing between 1.8 and 3.8 kg were anaesthetized by intravenous injection of pentobarbitone sodium (30 mg/kg) and the maximal twitches of the tibialis anterior muscle of the right hind limb were recorded in preparations similar to those described for cats.

Chicks

Rhode Island Red × Light Sussex chicks of either sex (1–4 weeks old) were intravenously injected with one or other of several neuromuscular blocking agents, and the type and duration of paralysis were recorded as described by Buttle & Zaimis (1949).

Blood levels of NN'-dimethylconessine

The concentrations of *NN'*-dimethylconessine in cat blood were determined by a biological method similar to that described for *d*-tubocurarine (Mahfouz, 1949). Samples of blood (1 ml.) were extracted with industrial methylated spirit (10 ml.) and the filtrate evaporated to dryness under reduced pressure. The residue was dissolved in frog Ringer solution and compared with a standard solution of *NN'*-dimethylconessine for its ability to antagonize contracture of the isolated rectus abdominis muscle of the frog induced by acetylcholine. Concentrations of 0.1 μg/ml. were assayable.

Because extracts of monkey blood caused contracture of the frog rectus muscle and potentiated the response induced by acetylcholine, they were assayed instead on the isolated semispinalis muscle of the chick (Child & Zaimis, 1960). Concentrations greater than 0.5 μg/ml. were assayable.

Drugs

Solutions of all drugs were prepared in physiological saline. Doses of drugs refer to the free base. *NN'*-dimethylconessine was available first as the diiodide; the dichloride which is more soluble in water was later prepared and used in some of the experiments. The steroidal mono and bis-quaternary ammonium compounds were prepared as a variety of salts as indicated in Tables 1 and 2 and were synthesized by our colleagues in the Chemistry Department of Glaxo Research Ltd.

RESULTS

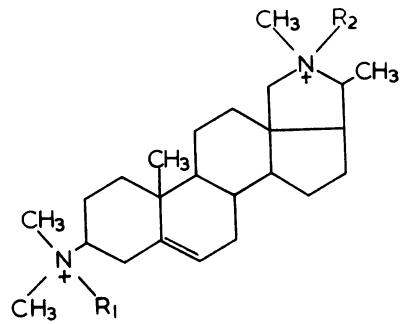
Neuromuscular blocking activities of mono and bis-quaternary ammonium derivatives of conessine

The activities of these compounds in cats are summarized in Table 1. The conessine derivatives were, almost without exception, potent, short-acting neuromuscular blocking agents of the non-depolarizing type. The neuromuscular blockade produced by intravenous injection of a dose sufficient to produce 95–100% block of the indirectly elicited tibialis twitch response was rapid in onset, of short duration and low cumulative propensity. The cumulative effect was assessed from the shortest interval in minutes necessary for repeated equal doses to produce equal effects which was defined as the

TABLE 1

STRUCTURES AND NEUROMUSCULAR BLOCKING ACTIVITIES OF MONO AND BIS-QUATERNARY AMMONIUM DERIVATIVES OF CONESSINE

Neuromuscular blocking activity was assessed in anaesthetized artificially respired cat preparations in which the twitch responses of the tibialis and soleus muscles were elicited by stimulation of the sciatic nerve once every 10 sec using square wave pulses of supramaximal strength and 0·2 msec duration. Potency refers to the dose in mg base/kg required to produce 95–100% block of the tibialis muscle. Cumulation time refers to the shortest interval in minutes required for repeated doses to produce equal effects. NT, Not tested. All drugs injected intravenously.



GR No.	Name	R_1	R_2	Number of cat experiments	Potency (mg base/kg)	Duration of block (min)		Cumulation time (min)	Block antagonized by neostigmine
						Tibialis	Soleus		
	(a) Mono-quaternary								
642	*N*-Methylconessine iodide	CH_3		2	2·97	5–10	10–20	30	+
653	*N*-Ethylconessine iodide	C_2H_5		2	1·13	15–20	15–20	60	+
657	*N*-*n*-Butylconessine iodide	C_4H_9		2	0·58	5–15	10–15	15–30	+
645	*N*-Chlormethylconessine bromide	CH_2Cl		2	1·70	10–20	15–30	60	+
652	*N*-Ethoxycarbonylmethylconessine bromide	$CH_2COOC_2H_5$		2	3·85	15–20	15–30	60	+
677	*N*-Benzylconessine bromide	$CH_2C_6H_5$		2	0·64	10–15	10–20	30	+
654	*N*-β-Phenoxyethylconessine bromide	$CH_2CH_2OC_6H_5$		2	0·83	5–15	10–20	15–30	+
656	Conessine mono-*N*-oxide	O		2	20·0	5–15	5–15	<60	NT

TABLE 1—continued

GR No.	Name	R_1	R_2	Number of cat experiments	Potency (mg base/kg)	Duration of block (min)		Cumulation time (min)	Block antagonized by neostigmine
						Tibialis	Soleus		
	(*b*) *Bis-quaternary*								
615	*NN'*-Dimethylconessine diiodide	CH_3	CH_3	7	0·60	5–20	10–20	30	+
632	*NN'*-Diethylconessine diiodide	C_2H_5	C_2H_5	3	0·47	10–20	10–20	30	+
646	*NN'*-Dipropylconessine diiodide	C_3H_7	C_3H_7	2	0·79	10–25	10–30	30–60	+
633	*NN'*-Diallylconessine dibromide	CH_2=$CHCH_2$	CH_2=$CHCH_2$	3	0·55	10–20	10–20	30	+
644	*NN'*-Dipropargylconessine dibromide	$CH_2C{\equiv}CH$	$CH_2C{\equiv}CH$	2	0·66	20–30	25–35	60	+
643	*NN'*-Dicrotylconessine dibromide	CH_2CH=$CHCH_3$	CH_2CH=$CHCH_3$	2	0·37	15–30	20–30	30–60	+
659	*NN'*-Diethoxycarbonylmethylconessine dibromide	$CH_2COOC_2H_5$	$CH_2COOC_2H_5$	2	0·38	20–30	20–30	NT	NT
661	*NN'*-Dimethoxyethylconessine diiodide	$CH_2CH_2OCH_3$	$CH_2CH_2OCH_3$	2	0·82	10–20	15–20	30–60	+
641	*NN'*-Dibenzylconessine diiodide	$CH_2C_6H_5$	$CH_2C_6H_5$	2	0·72	20–30	25–35	60–90	−
681	*N'*-Methylconessine chloride hydrochloride	H	CH_3	2	1·05	10–15	15–20	30–90	+
685	*N*-Isopropyl-*N'*-methylconessine diiodide	$CH(CH_3)_2$	CH_3	2	0·23	15–20	15–25	60	+
658	*N*-*n*-Butyl-*N'*-methylconessine diiodide	C_4H_9	CH_3	2	0·24	10–15	10–15	30–90	+
686	*N*-Octyl-*N'*-methylconessine diiodide	C_8H_{17}	CH_3	2	1·47	10–20	10–15	15–30	+
650	*N*-Chlormethyl-*N'*-methylconessine bromide iodide	CH_2Cl	CH_3	2	0·52	15–30	20–30	30–60	+
655	*N*-Methoxy-*N'*-methylconessine diiodide	OCH_3	CH_3	2	0·61	10–15	15–25	30–60	+
684	*N*-Benzyl-*N'*-methylconessine dichloride	$CH_2C_6H_5$	CH_3	2	0·34	10–20	10–20	30–60	+
671	*N*-β-Phenoxyethyl-*N'*-methylconessine bromide iodide	$CH_2CH_2OC_6H_5$	CH_3	2	0·27	5–15	5–20	30–60	+
667	*N*-Methoxymethylconessine chloride hydrochloride	CH_2OCH_3	H	2	1·74	5–15	10–15	30–60	+

TABLE 2

STRUCTURES AND NEUROMUSCULAR BLOCKING ACTIVITIES OF MISCELLANEOUS STEROIDAL MONO AND BIS-QUATERNARY AMMONIUM SALTS

Neuromuscular blocking activity was assessed in anaesthetized artificially respired cat preparations in which the twitch responses of the tibialis and soleus muscles were elicited by stimulation of the sciatic nerve once every 10 sec using square wave pulses of supramaximal strength and 0·2 msec duration. Potency refers to the dose in mg base/kg required to produce 95–100% block of the tibialis muscle. Cumulation time refers to the shortest interval in minutes required for repeated doses to produce equal effects. NT, Not tested. All drugs injected intravenously.

GR No.	Name	Number of cat experiments	Potency (mg base/kg)	Duration of action (min)		Cumulation time (min)	Block antagonized by neostigmine
				Tibialis	Soleus		
	(a) Mono-quaternary						
679	Cona-3-5-dienine methiodide	1	>10·8				NT
672	20α-Dimethylamino-5α-pregnan-3β-ol. methiodide	2	>7·4				NT
676	20α-Dimethylamino-5α-pregnan-3β-one methiodide	1	>7·4				NT
675	3β-Acetoxy-20α-dimethylamino-5α-pregnane methiodide	1	>6·1				NT
	(b) Bis quaternary						
616	*NN'*-Dimethyldihydroconessine diiodide	3	0·45	10–20	10–20	30	+
648	*NN'*-Dimethylheteroconessine diiodide	1	0·60	20	20	30	+
647	*NN'*-Diallyldihydroconessine dibromide	2	0·38	10–20	15–25	30–45	+
673	3β-Dimethylamino-cona-4-6-dienine bismethiodide	2	0·61	5–20	10–20	60–120	+
678	3β-Dimethylamino-conanine-5α,6α-epoxide bismethiodide	2	0·79	30–60	30–60	60–120	+
674	3β-Dimethylamino-conanine-5β,6β-epoxide bismethiodide	3	0·62	5–30	10–35	30–60	+
663	3β-Dimethylamino-6-oxo-conanine bismethiodide	3	0·31	15–40	20–40	60–120	+
664	3β-Dimethylamino-5,6-dihydroxyconanine bismethiodide	2	1·88	15–30	15–30	60	+
666	3β-Dimethylamino-6α-hydroxyconanine bismethiodide	3	0·41	15–20	15–20	60–120	+
682	3β-Dimethylamino-5-hydroxy-con-6-enine-acetate methiodide hydriodide	2	0·96	5–15	10–20	60	+
662	3β-Dimethylamino-5-hydroxy-con-6-enine methiodide hydriodide	2	1·05	15–25	15–30	60–120	+
680	3β-Dimethylamino-con-*N*-20-eninium chloride methochloride	2	0·32	10–20	15–25	90–120	+
665	3β-Dimethylamino-con-*N*-20-eninium chloride hydrochloride	2	2·10	5–25	5–25	60–120	+
689	1,6-Hexamethylene-bis[3*N*] conessinium diiodide	1	0·11	50	60	90	+
688	1,10-Decamethylene-bis[3*N*] conessinium diiodide	2	0·06	40	55	>120	+
683	Tetrahydroconessimethine bismethiodide	2	0·18	10–20	10–20	120	+
690	3β,-24-Bisdimethylamino-chol-5-ene bismethochloride	2	0·33	10–15	10–15	15–30	–
670	3β,20α-Bisdimethylamino-5α-pregnane methiodide (Malouétine)	2	0·45	5–15	5–15	15–30	+

cumulation time. There was no initial potentiation of the twitch and no muscle fasciculation. The block could be rapidly antagonized by administration of neostigmine.

In the 3*N*-monoquaternary compounds, potency increased in the order methyl<ethyl< butyl and, with one exception, substitution in the methyl and ethyl groups also increased activity. Potency was markedly reduced, however, when the cation was differently constituted (compound GR-656).

The properties of all the bis-quaternary ammonium derivatives of conessine were uniform, and were almost identical with those of *NN'*-dimethylconessine (GR-615). The *NN'*-dibenzyl-compound (GR-641), however, produced neuromuscular block which could not be antagonized by repeated injections of neostigmine bromide (37 and 74 μg/kg), although all other features of the block were curare-like. This compound resembles benzoquinonium, which is similar to *d*-tubocurarine in its mechanism of action but cannot be antagonized by anticholinesterases (Bowman, 1958).

Neuromuscular blocking activities of miscellaneous steroidal mono and bis-quaternary ammonium compounds

The activities of these compounds are summarized in Table 2. The 20*N*-monoquaternary compound GR-679 and the mono-quaternary pregnane compounds GR-672, -676 and -675 were less active than the compounds in the previous series. With the bis-quaternary steroids, the potency and block duration for compounds GR-673, -678, -674, -663, -664, -666, -682, -662, -680 and -665 were similar to those obtained with the *NN'*-bis-quaternary conessine compounds but the cumulation time was consistently increased. The bis-[3*N*]-conessinium compounds GR-689 and GR-688 were the most potent neuromuscular blocking agents tested but these compounds were also cumulative. The neuromuscular block produced by GR-690 could not be antagonized with neostigmine. The steroidal alkaloid malouétine, previously examined by Janot, Lainé & Goutarel (1960), and included here as compound GR-670, possessed potency and block characteristics similar to those obtained with the conessine series.

Pharmacology of NN'-dimethylconessine

1. Neuromuscular blocking activity. In the cat, *NN'*-dimethylconessine produced neuromuscular block qualitatively similar to that produced by administration of *d*-tubocurarine; the drugs differed little in potency but considerably in duration of action and cumulation. Small doses of *NN'*-dimethylconessine (0.15 mg/kg) produced a 10–20% block of the indirectly elicited tibialis twitch and a similar or slightly greater block of the soleus twitch; with 0.6 mg/kg, both muscles were completely paralysed within 40 sec of injection, complete block in the tibialis lasting for about 2 min. The tibialis and soleus muscles exhibited a slight difference in sensitivity to the drug; usually the soleus was more affected, as has been observed with *d*-tubocurarine (Paton & Zaimis, 1951). Once started, recovery was rapid, the twitch tension returning to its pre-injection level in 5–20 min (Fig. 1d). The duration of action and cumulation time for *NN'*-dimethylconessine were similar to those obtained with the short-acting depolarizing drug succinylcholine (Table 3). With both compounds, intravenous administration of small doses, each time the twitch response returned to normal, did not result in cumulation; similar administration of gallamine triethiodide resulted in a greater block and

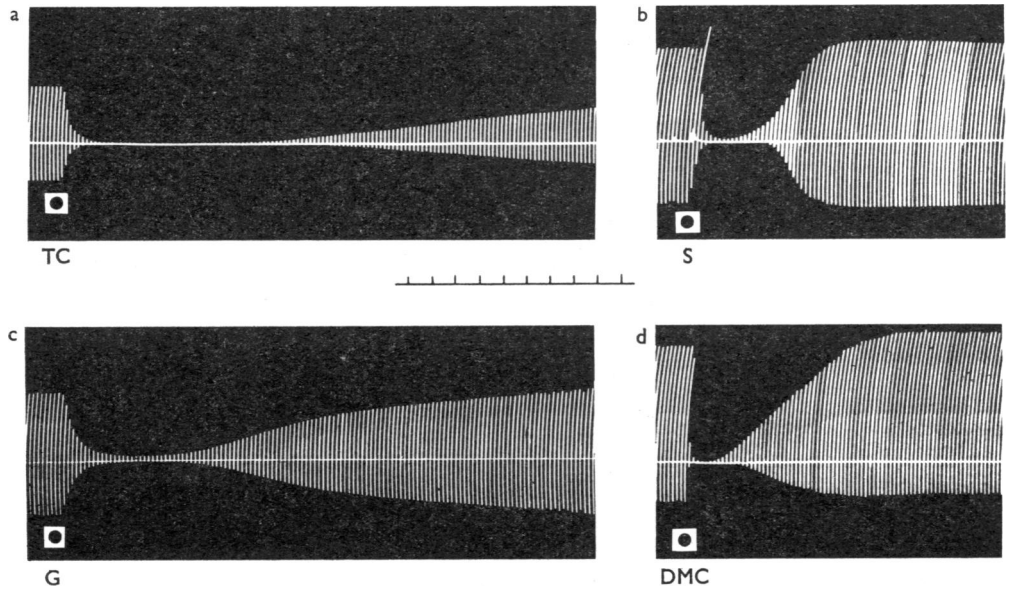


Fig. 1. Effects of single intravenous doses of neuromuscular blocking drugs on the response of the tibialis muscle of the cat. Maximal twitches of the tibialis anterior muscle were elicited indirectly once every 10 sec. Time marker indicates minute intervals. (a) Cat 2.6 kg. At TC, *d*-tubocurarine 0.45 mg/kg was administered. (b) Cat 3.4 kg. At S, succinylcholine 0.064 mg/kg. (c) Cat 4.2 kg. At G, gallamine triethiodide 1.0 mg/kg. (d) Cat 4.2 kg. At DMC, *NN'*-dimethylconessine 0.30 mg/kg.

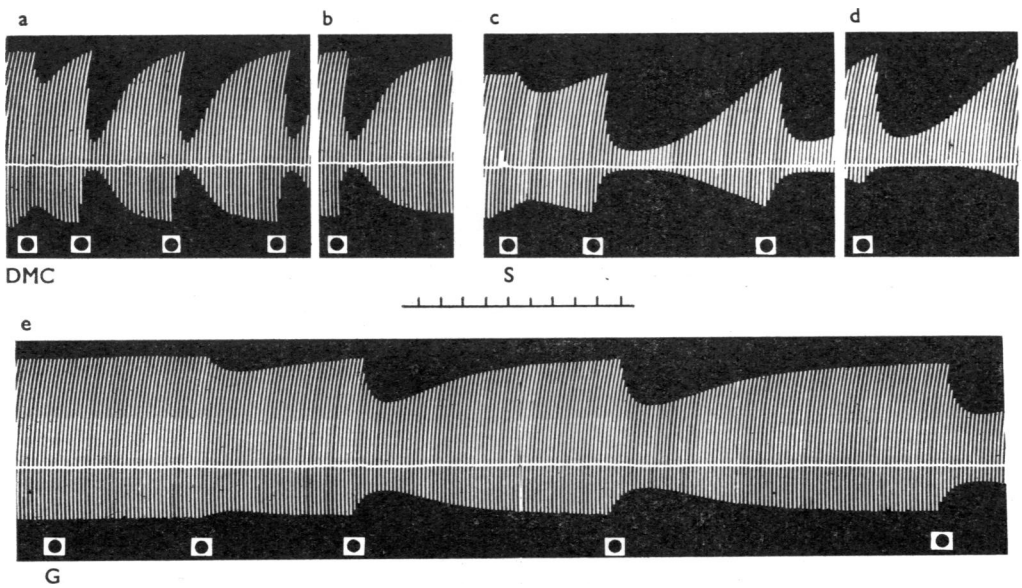


Fig. 2. Effects of repeated intravenous doses of neuromuscular blocking drugs on the response of the tibialis muscle of the cat. Maximal twitches of the tibialis anterior muscle were elicited indirectly once every 10 sec. Doses were repeated each time the tibialis twitch tension returned to its pre-injection level. Time marker indicates minute intervals. (a) and (b) Cat 4.8 kg. Ten doses of *NN'*-dimethylconessine 0.15 mg/kg (DMC) were injected. The responses to doses 5 to 9 inclusive have been omitted between (a) and (b). (c) and (d) Cat 3.2 kg. Ten doses of succinylcholine 0.016 mg/kg (S) were given ; the responses to doses 4 to 9 inclusive have been omitted between (c) and (d). (e) Same cat as in (a) after an interval of 60 min. Five doses of gallamine triethiodide 0.5 mg/kg (G) were given.

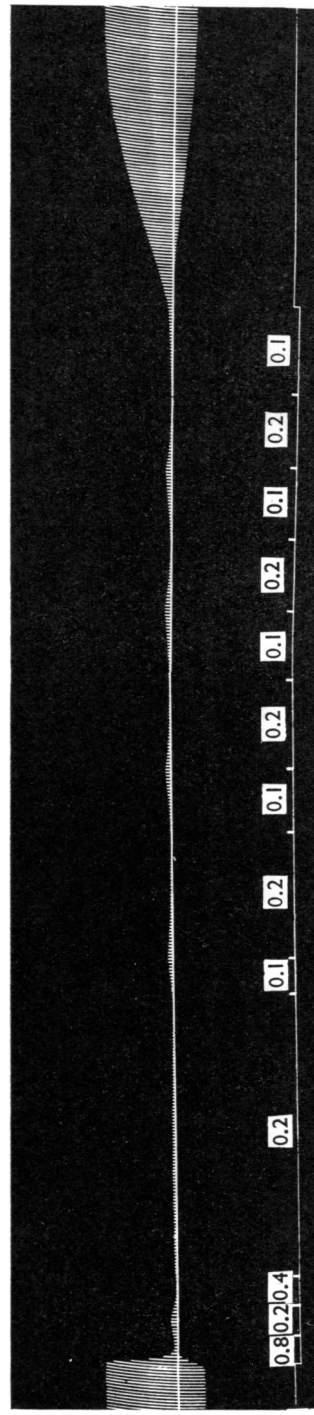


Fig. 3. Cat 3.4 kg. Effects of intravenous infusion of *NN'*-dimethylconessine on the response of tibialis muscle of the cat. Maximal twitches of the tibialis anterior muscle were elicited indirectly once every 10 sec. Sufficient *NN'*-dimethylconessine was infused to maintain a 90–95% block of the twitch response. Numbers refer to the infusion rate in mg/kg/min. Time marker indicates minute intervals.

TABLE 3

COMPARISON OF NEUROMUSCULAR BLOCKING AGENTS IN THE CAT

Neuromuscular blocking activity was assessed in anaesthetized artificially respired cat preparations in which the twitch responses of the tibialis and soleus muscles were elicited by stimulation of the sciatic nerve once every 10 sec using square wave pulses of supra-maximal strength and 0·2 msec duration. Potency refers to the dose in mg base/kg required to produce 95–100% block of the tibialis muscle. Cumulation time refers to the shortest interval in minutes required for repeated doses to produce equal effects.

Drug	Number of cat experiments	Potency (mg base/kg)	Duration of block (min)		Cumulation time (min)
			Tibialis	Soleus	
d-Tubocurarine dichloride	2	0·37	30–50	30–60	120–180
Gallamine triethiodide	2	0·86	20–30	25–40	120
Succinylcholine dichloride	2	0·062	5–10	5–10	15–30
NN'-Dimethylconessine (diiodide or dichloride)	9	0·60	5–20	10–20	30

longer recovery time with each successive dose (Fig. 2). A 90–95% block of the indirectly elicited tibialis twitch was easily maintained when *NN'*-dimethylconessine was infused intravenously (Fig. 3); when insufficient was infused to produce block, a slight increase in twitch tension was observed. This transient stimulating action was similar to that obtained with small doses of other competitive antagonists in the cat (Payne, 1961).

The paralysis produced by *NN'*-dimethylconessine was localized at the neuromuscular junction; thus the response of the tibialis muscle to indirect stimulation was completely prevented while the response to direct stimulation was unaffected. Similarly the gross muscle action-potential was abolished after injection of *NN'*-dimethylconessine whereas the antidromic nerve action-potential remained unchanged (Fig. 4). *NN'*-dimethyl-

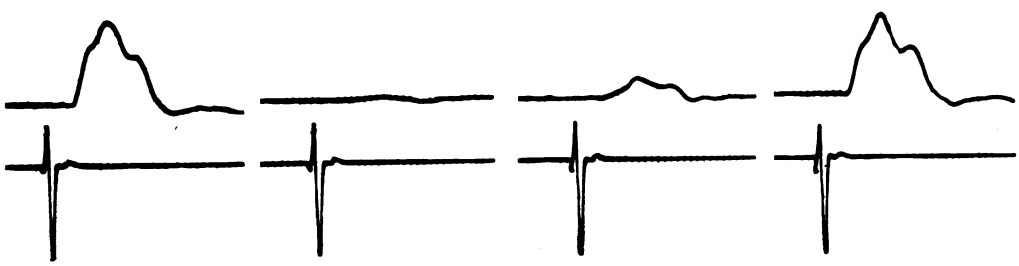


Fig. 4. Cat 2.8 kg. Effect of a single intravenous dose of *NN'*-dimethylconessine on the electrical recordings from the tibialis muscle and sciatic nerve of the cat. Maximal responses of the tibialis anterior muscle were elicited indirectly once every 10 sec. Upper traces: gross muscle action-potentials recorded from platinum electrodes inserted in the tibialis muscle. Lower traces: compound nerve action-potentials recorded antidromically from platinum electrodes placed on the sciatic nerve,. The recordings (left to right) were obtained immediately before and 1, 10 and 20 min after the intravenous injection of *NN'*-dimethylconessine (0.60 mg/kg).

conessine, like curare, blocks by competitive antagonism of acetylcholine; thus 120 μg injected close-arterially to the tibialis muscle in a cat reduced the muscle response to indirect stimulation and abolished the response to close-arterial injection of acetylcholine (Fig. 5). During partial block produced by injection of *NN'*-dimethylconessine, tetanic stimulation of the motor nerve caused a brief twitch-like response in the tibialis muscle which was not sustained and which was followed by potentiation of the indirectly elicited twitch; during block with succinylcholine the tetanic tension was reduced but well sustained and post-tetanic potentiation did not occur (Fig. 6). Neuromuscular block

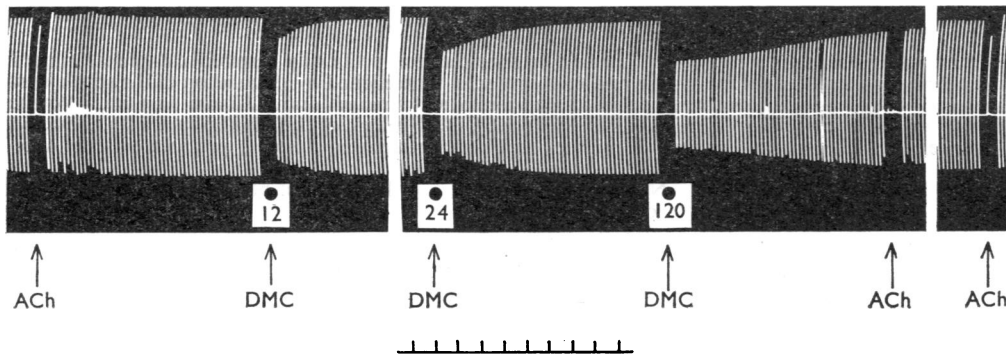


Fig. 5. Cat 3.5 kg. Effects of close-arterial injection of *NN'*-dimethylconessine on the response of the tibialis muscle of the cat. Maximal twitches of the tibialis anterior muscle were elicited indirectly once every 10 sec. Time marker indicates minute intervals. At ACh, electrical stimulation was stopped and 50 μg acetylcholine was injected close-arterially. At DMC, *NN'*-dimethylconessine was injected close-arterially in doses of 12, 24 and 120 μg.

produced by *NN'*-dimethylconessine was rapidly and completely antagonized by injection of neostigmine (37 μg/kg). The potency and duration of action of *NN'*-dimethylconessine were increased two to four times when cats were exposed to anaesthetic concentrations of ether or halothane. The increase depended on the concentration and period of administration of the volatile anaesthetic.

NN'-dimethylconessine injected into the pectoral vein of chicks in a dose of 0.22 mg/kg produced a flaccid paralysis similar to that produced by the injection of

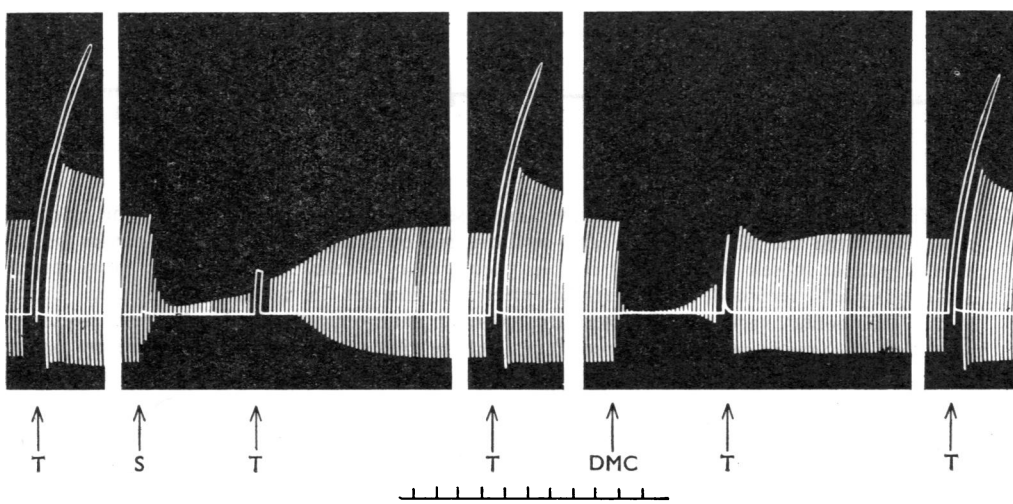


Fig. 6. Cat 3.2 kg. Effects of succinylcholine and *NN'*-dimethylconessine on the response of the tibialis muscle of the cat to tetanic stimulation of the sciatic nerve. Maximal twitches of the tibialis anterior muscles were elicited indirectly once every 10 sec. Time marker indicates minute intervals. At T, tetanic stimulation of the sciatic nerve was maintained for 15 sec at a frequency of 50/sec. As S, succinylcholine 0.032 mg/kg was injected intravenously. At DMC, *NN'*-dimethylconessine 0.60 mg/kg was injected intravenously.

d-tubocurarine 0.45 mg/kg or gallamine triethiodide 0.14 mg/kg and quite different from the spastic paralysis produced by the injection of decamethonium 0.067 mg/kg.

In monkeys anaesthetized with pentobarbitone sodium, complete block of the indirectly elicited tibialis twitch was produced by intravenous administration of *NN'*-dimethylconessine 0.30 mg/kg. In contrast to the results obtained in cats, the block lasted about 1 hr in monkeys and at least another hour had to elapse before successive doses produced a similar response. Block was readily reversed by the administration of neostigmine.

2. *Cardiovascular activity.* A slight transient fall in blood pressure sometimes occurred in cats after intravenous administration of *NN'*-dimethylconessine 0.60 mg/kg. With higher doses (3.0–6.0 mg/kg), the depressor responses were greater (40–60 mm Hg) and lasted for 5–10 min. The fall in blood pressure, which was partially prevented by pre-treatment with mepyramine maleate (2.0 mg/kg, given in divided doses) was accompanied by a reduction in the response of the nictitating membrane to preganglionic stimulation and a reduction of the cardiovascular responses to peripheral vagal stimulation. The responses of the cardiovascular system to acetylcholine, histamine and adrenaline were unaffected by these doses of *NN'*-dimethylconessine. The electrocardiogram (lead II) was not altered with doses up to 6.0 mg/kg. The monkey was much less sensitive to the hypotensive action of *NN'*-dimethylconessine; doses of 15 mg/kg produced a gradual fall in blood pressure of about 40 mm Hg.

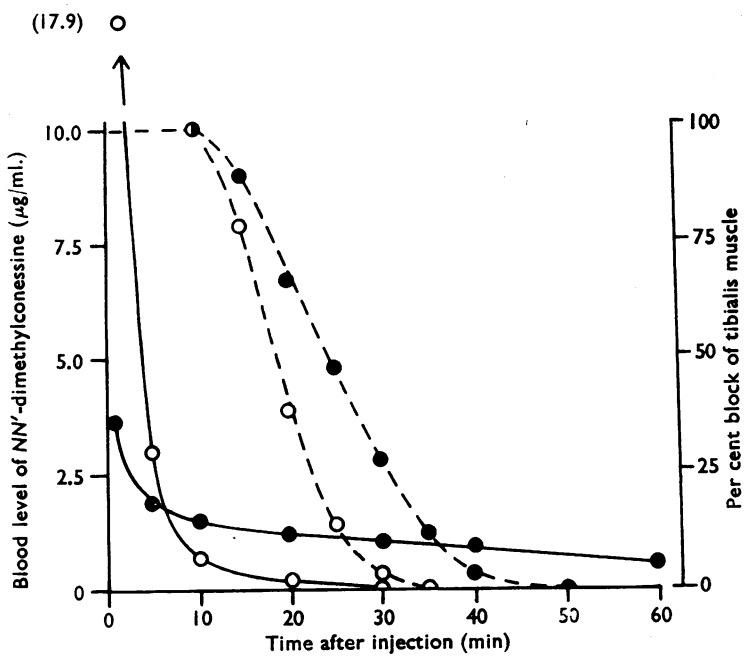


Fig. 7. Relationship between neuromuscular block and blood level of *NN'*-dimethylconessine in anaesthetized artificially respired cats and monkeys. The percentage block of the indirectly elicited tibialis twitch (broken lines) and blood level of *NN'*-dimethylconessine (continuous lines) are shown at various times after the intravenous injection of *NN'*-dimethylconessine. Cats (open circles) received 3.0 mg/kg and monkeys (closed circles) 0.6 mg/kg respectively. Results are the averages from three cat and two monkey experiments.

3. Relationship between neuromuscular block and blood level of NN'-dimethylconessine. An attempt was made to find the reason for the difference in the duration of the block observed in the cat and the monkey by determining the blood concentrations of *NN'*-dimethylconessine during the period of neuromuscular blockade in the two species. Doses were employed which produced a similar duration of block of the indirectly elicited tibialis twitch. The results (Fig. 7) indicate that *NN'*-dimethylconessine is removed less quickly from the blood of the monkey than from that of the cat and it is conceivable that the drug persists longer in effective concentrations at the receptor sites in the monkey.

DISCUSSION

The muscle relaxant drugs which are at present available possess disadvantages and the need for short-acting curare-like compounds has been repeatedly stated (Foldes, 1957; Churchill-Davidson, 1958; Bowman, 1962). Attempts to produce such compounds have been made (Haining, Johnston & Smith, 1959; Cheymol, Guidicelli, Chabrier & Najer, 1959; Brittain, Collier & D'Arcy, 1961; Khuong Huu-Lainé & Pinto-Scognamiglio, 1964; Biggs, Davis & Wien, 1964).

The potency, duration of block and type of neuromuscular block obtained with these new steroidal compounds (Tables 1 and 2) indicated that many might possess the desired clinical properties. *NN'*-dimethylconessine was selected for clinical trial because its block duration and cumulative propensity in the cat, an animal generally acknowledged to resemble man most closely in its sensitivity to neuromuscular blocking drugs (Bowman, 1964), were as short as those obtained with succinylcholine and a similar short duration of action and lack of cumulation were obtained with experiments on conscious rabbits (unpublished observations).

In man, *NN'*-dimethylconessine caused rapid onset of a non-depolarizing neuromuscular block without change in blood pressure or heart rate and without the development of secondary curarization or post-operative muscle pains (Verner, 1963). The duration of effect of a full paralysing dose in man (0.30 mg/kg), however, as judged by the time required for 50% recovery of the developed muscle tension produced by percutaneous stimulation, was three times longer than with a corresponding dose of succinylcholine.

The results obtained by Verner (1963) in man correspond more closely with our findings in the monkey, at least with respect to duration of action. This confirms the suggestion made by Mapleson & Mushin (1964) that the monkey may be a more suitable animal than the cat for predicting recovery times of new steroidal muscle relaxant drugs in man. Mapleson & Mushin found that the duration of action in man of dipyrandium chloride, 3β,17β-dipyrrolidin-1'-yl-5α-androstane bismethochloride (M & B 9105A), more closely resembled the recovery times obtained in monkeys by Biggs *et al.* (1964), although the latter workers had shown that dipyrandium had a similar duration of action to succinylcholine in the cat, rabbit and chicken.

The neuromuscular blocking action of other steroidal onium compounds has been described (Quevauviller & Lainé, 1960; Biggs *et al.*, 1964; Khuong Huu-Lainé & Pinto-Scognamiglio, 1964; Alauddin, Caddy, Lewis, Martin-Smith & Sugrue, 1965; Bamford, Biggs, Davis & Parnell, 1967; Lewis, Martin-Smith, Muir &

Ross, 1967). Stenlake (1963) has drawn attention to the rigidity of the steroid nucleus in relation to biological activity, and Alauddin *et al.* (1965) investigated their series of bis-quaternary androstane compounds specifically because of the relatively inflexible configuration of the nucleus. Alauddin *et al.* (1965) concluded that a "fixed" interonium distance was relatively unimportant in determining neuromuscular blocking activity, a conclusion also reached by Bamford *et al.* (1967) who investigated a series of stereoisomeric androstane 3,17-bisquaternary salts. Bamford *et al.* (1967) have suggested 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 potency.

The bis-quaternary compounds reported here (Table 1) all possess an identical interonium distance (10.1Å, Dreiding stereomodels), an inflexible pentacyclic steroidal nucleus and similar potency and duration of action. It is significant that changing the quaternizing groups in both the symmetrical and asymmetrical bis-quaternary compounds exerted little effect on potency or block duration and only in the case of compound GR-641 (*NN'*-dibenzylconessine) was the type of block affected. Seven of the eight 3*N*-monoquaternary compounds tested were also potent neuromuscular blocking drugs producing a typical curare-like response of short duration. The relative inactivity of conessine mono-*N*-oxide (GR-656) may be attributed to the different constitution of this particular cation head.

The similarity in activity of these particular mono- and bis-quaternary steroids supports the one-point attachment theory of Loewe & Harvey (1952). In *NN'*-dimethylconessine the cation head attached to the 3-position is in the same plane as rings A, B, C and D of the steroid nucleus; both the double bond at 5,6 and the possibility of steric hindrance between the 3*N*-methyl groups and the angular group at carbon-10 confer the "chair" configuration on rings A and B of the conessine skeleton. Ring E, which contains the second nitrogen at position "20," is at right angles to the plane of the cyclopentanoperhydrophenanthrene nucleus and, if the one-point attachment theory is correct, it could be expected to point away from the receptor surface and therefore be without influence on activity.

SUMMARY

1. The neuromuscular blocking activities of a series of mono and bis-quaternary ammonium salts of conessine and other related steroids have been examined on cat nerve-muscle preparations.

2. In the cat most of these compounds possessed short-acting muscle relaxant properties of the non-depolarizing type; block duration was shorter, and cumulation was less than with gallamine or *d*-tubocurarine.

3. The structure-activity relationships of these rigid steroidal mono and bis-quaternary ammonium compounds are discussed briefly in relation to the theories of drug-receptor interaction.

4. The nature of the quaternizing groups in the bis-quaternary conessine compounds exerted little effect on potency or block duration; moreover mono-quaternary conessine derivatives possessed similar activities to those obtained with the bis-onium compounds.

5. The pharmacology of one compound, *NN'*-dimethylconessine, whose muscle relaxant properties are typical of many of the compounds in the conessine series and which has been tested clinically, was studied in detail.

6. *NN'*-dimethylconessine was longer acting in the monkey than in the cat, and it was concluded from the results of the clinical trial of this compound that the duration of effect in the monkey is of greater predictive value in testing steroidal neuromuscular blocking drugs.

REFERENCES

ALAUDDIN, M., CADDY, B., LEWIS, J. J., MARTIN-SMITH, M. & SUGRUE, M. F. (1965). Non-depolarizing neuromuscular blockade by 3α,17α-bis(quaternary ammonium) 5α-androstanes. *J. Pharm. Pharmac.*, **17**, 55–59.

BAMFORD, D. G., BIGGS, D. F., DAVIS, M. & PARNELL, E. W. (1967). Neuromuscular blocking properties of stereoisomeric androstane-3,17-bisquaternary ammonium salts. *Br. J. Pharmac. Chemother.*, **30**, 194–202.

BERTHO, A. (1944). Kurchi-Alkaloide. III. Über das wirksame Hauptalkaloid Conkurchin. *Annalen*, **555**, 214–240.

BIGGS, R. S., DAVIS, M. & WIEN, R. (1964). Muscle relaxant properties of a steroid bis-quaternary ammonium salt. *Experientia*, **20**, 119–120.

BOWMAN, W. C. (1958). The neuromuscular blocking action of benzoquinonium chloride in the cat and hen. *Br. J. Pharmac. Chemother.*, **13**, 521–530.

BOWMAN, W. C. (1962). Mechanisms of neuromuscular blockade. *Prog. mednl Chem.*, **2**, 88–131.

BOWMAN, W. C. (1964). Neuromuscular blocking agents. *Evaluation of drug activities* : *Pharmacometrics*, vol. 1, pp. 325–351. London : Academic Press.

BRITTAIN, R. T., COLLIER, H. O. J. & D'ARCY, P. F. (1961). The neuromuscular blocking action of γ-oxalolaudonium bromide. *Br. J. Pharmac. Chemother.*, **17**, 116–123.

BROWN, G. L. (1938). The preparation of the tibialis anterior (cat) for close arterial injections. *J. Physiol., Lond.*, **92**, 22*P*.

BURN, J. H. (1914). The action of conessine and holarrhenine, the alkaloids of *Holarrhena congolensis*, and also of oxyconessine. *J. Pharmac. exp. Ther.*, **6**, 305–321.

BUTTLE, G. A. H. & ZAIMIS, E. (1949). The action of decamethonium iodide in birds. *J. Pharm. Pharmac.*, **1**, 991–992.

CHEYMOL, J., GUIDICELLI, R., CHABRIER, P. & NAJER, H. (1959). Étude pharmacologique d'un nouveau curarimimétique à action brève. *C.r. hebd. Séanc. Acad. Sci., Paris*, **248 II**, 1723–1725.

CHILD, K. J. & ZAIMIS, E. (1960). A new biological method for the assay of depolarizing substances using the isolated semispinalis muscle of the chick. *Br. J. Pharmac. Chemother.*, **15**, 412–416.

CHURCHILL-DAVIDSON, H. C. (1958). The muscle relaxants. *Br. med. Bull.*, **14**, 31–33.

FOLDES, F. F. (1957). *Muscle Relaxants in Anaesthesiology*, Springfield, Illinois : Charles C. Thomas.

HAINING, C. G., JOHNSTON, R. G. & SMITH, J. M. (1959). Neuromuscular blocking agents of short duration. *Nature, Lond.*, **183**, 542–543.

JANOT, M., LAINÉ, F. & GOUTAREL, R. (1960). Alcaloides steroides *V* : Alcaloides du *Malouetia bequaertiana* E. Woodson (Apocynacées) : La funtuphyllamine B et la malouétine. *Annls pharm. fr.*, **18**, 673–677.

KHUONG HUU-LAINÉ, F. & PINTO-SCOGNAMIGLIO, W. (1964). Activité curarisante du dichlorure de 3β, 20α-bistrimethylammonium 5α-pregnane (malouétine) et de ses stéreoisomères. *Archs int. Pharmacodyn. Thér.*, **147**, 209–219.

LEWIS, J. J., MARTIN-SMITH, M., MUIR, T. C. & ROSS, H. H. (1967). Steroidal monoquaternary ammonium salts with non-depolarizing neuromuscular blocking activity. *J. Pharm. Pharmac.*, **19**, 502–508.

LOEWE, S. & HARVEY, S. C. (1952). Equidistance concept and structure-activity relationship of curarizing drugs. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **214**, 214–226.

MAHFOUZ, M. (1949). The fate of tubocurarine in the body. *Br. J. Pharmac. Chemother.*, **4**, 295–303.

MUSHIN, W. W. & MAPLESON, W. W. (1964). Relaxant action in man of dipyrandium chloride (M & B 9105A). (A steroid bis-quaternary ammonium salt.) *Br. J. Anaesth.*, **36**, 761–768.

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

PAYNE, J. P. (1961). The initial transient stimulant action of neuromuscular blocking agents in the cat. *Br. J. Anaesth.*, **33**, 285–288.

POLSTORFF, K. & SCHIRMER, P. (1886). Ueber Conessin. *Chem. Ber.*, **19**, 78–85.

QUEVAUVILLER, A. & LAINÉ, F. (1960). Sur la toxicité et le pouvoir curarisant du chlorure de malouétine. *Annls. pharm. fr.*, **18**, 678–680.

STENLAKE, J. B. (1963). Some chemical aspects of neuromuscular block. *Prog. mednl. Chem.*, **3**, 1–51.

STEPHENSON, R. P. (1948). The pharmacological properties of conessine, isoconessine and neoconessine. *Br. J. Pharmac. Chemother.*, **3**, 237–245.

VERNER, I. R. (1963). Some problems in the clinical evaluation of relaxant drugs in man, with special reference to a new competitive agent. Communication to the Section of Anaesthetics, Royal Society of Medicine, April 5, 1963.