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The neuromuscular and autonomic blocking activities of pancuronium, Org NC 45, and other pancuronium analogues, in the cat

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Twenty-six mono- or bis-quaternary salts of 3,17-dioxy-2β,16β-dipiperidino-5α-androstanes (including pancuronium) and one 17-desoxy congener were tested for neuromuscular blocking and autonomic blocking activities in the chloralose-anaesthetized cat. The 17β-acetoxy series, all the members of which contain an acetylcholine-like fragment in the steroidal D-ring, was most selective for effecting neuromuscular blockade. The salient member of this series is 3α,17β-diacetoxy-2β,16β-dipiperidino-5α-androstane 16β-*N*-monomethobromide (Org NC 45) which is highly selective in blocking neuromuscular transmission in that a dose approximately sixty times greater than the neuromuscular blocking dose was required to block responses to vagal stimulation. In contrast, in doses sufficient to produce neuromuscular block, pancuronium simultaneously blocked responses to vagal stimulation. Moreover, pancuronium and Org NC 45 exhibited the same order of neuromuscular blocking activity and therefore the latter potentially represents a useful addition to the armamentarium of neuromuscular blocking agents currently in clinical use.

The steroid nucleus has often been used as an almost rigid framework for supporting quaternary ammonium functions in the design of non-depolarizing neuromuscular blocking drugs (Quevauviller & Lainé 1960; Allaudin et al 1965; Bamford et al 1967; Marshall et al 1973; Gandiha et al 1975). Some of these compounds have undergone clinical trial (Mushin & Mapleson 1964; Baird & Reid 1967; Feldman & Tyrrell 1970; Baird 1974), but only pancuronium has achieved widespread clinical use. The other compounds have either exhibited a long duration of action in man or unacceptable cardiovascular side effects, particularly a tendency to produce tachycardia.

Previous studies have suggested that the high neuromuscular blocking and low ganglion blocking potencies of pancuronium are associated with the molecular geometries and electronic structures of the acetylcholine-like fragments in the molecule (Savage et al 1971; Buckett et al 1973). Therefore the ester and *N*-alkyl substituents in these acetylcholine-like molecular fragments of pancuronium were varied and the resultant neuromuscular and autonomic blocking activities studied in an attempt to produce drugs which are more selective for their action on the neuromuscular junction than the standard agents in anaesthetic practice. This study allows some tentative conclusions to be drawn with respect to relating certain structural features in the pancuronium series to selectivity of pharmacological action.

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METHODS

Chick isolated biventer cervicis muscle preparation

Chicks aged 7 to 10 days were killed with ether and both the biventer cervicis muscles were dissected out (Ginsborg & Warriner 1960), mounted in organ baths containing Krebs–Henseleit (1932) solution at 32 °C and bubbled with 5% CO_2 in oxygen.

The muscles were stimulated indirectly via the motor nerve in the tendon of the muscle with ring electrodes. The stimulus consisted of rectangular pulses of a duration of 0·2 ms and at a frequency of 0·1 Hz, and of a strength greater than that required to elicit a maximal twitch response.

Anaesthetized cats

Cats of either sex, 1·6 to 3·7 kg, were anaesthetized with a mixture of α-chloralose (80 mg kg^{-1}) and pentobarbitone sodium (4·8 mg kg^{-1} injected i.p. The cats were artificially ventilated with 18 ml kg^{-1} of air at a rate of 26 breaths min^{-1}.

Drugs were administered i.v. through a polythene cannula placed in a femoral vein. Femoral arterial blood pressure was recorded via a polythene cannula connected to a Statham P23AC pressure transducer. The pulse pressure was also used to trigger a Grass

7P4F EKG tachograph or a Devices 2750 instantaneous ratemeter which measured heart rate.

Both vagus nerves were separated from the cervical sympathetic nerves and ligated. One vagus nerve was stimulated by trains of impulses (0·5 ms pulse duration at a frequency of 10 Hz for 10 s every 100 s) applied on the cardiac side of the ligature. The stimulation strength was adjusted to produce a constant decrease in heart rate of approximately 50%. In some experiments acetyl-β-methylcholine (7–20 μg kg^{-1}) was injected in a dose that produced a bradycardia approximately equal to that produced by vagal stimulation.

The cervical sympathetic nerves were ligated preganglionically and stimulated unilaterally with trains of impulses (0·5 ms pulse duration at a frequency of 10 Hz for 10 s every 100 s). The stimulus strength was adjusted to produce a maximal contraction of the nictitating membrane. In some experiments, the contralateral cervical nerve was ligated postganglionically and stimulation was applied postganglionically with the same stimulation parameters as those used for preganglionic stimulation. Contractions of the nictitating membranes were recorded by Grass FT03C force displacement transducers or UF1 2 oz strain gauges.

The sciatic nerve in the popliteal space was stimulated by rectangular pulses of 0·2 ms duration at a frequency of 0·1 Hz. The stimulation strength was adjusted to evoke maximal twitches of the tibialis anterior and soleus muscles. Contractions of these muscles were recorded by Grass FT10C force displacement transducers or UF1 32 oz strain gauges.

Dose-inhibition curves for the antagonists on the responses of the soleus muscle to sciatic nerve stimulation, of the heart rate to vagal stimulation, and of the nictitating membrane to preganglionic stimulation were constructed by injecting increasing doses of the antagonists. At least 60 min was allowed after full recovery of all parameters measured following injection of one dose of antagonist before a subsequent dose was injected.

Neuromuscular blockade during a drug effect was measured as the percentage of depression of twitch tension relative to the height of the control twitch responses. Onset time of neuromuscular blockade was measured as the time from injection to maximum depression of twitch tension, and recovery time was measured as the time from 25% to 75% of control responses.

Statistics

Each compound was tested initially in one cat. Compounds which possessed a neuromuscular blocking potency (the dose which produced 50% depression of twitch tension) which was greater than one tenth of that of pancuronium on the indirectly stimulated soleus muscle were tested in at least four cats. Regression analyses for neuromuscular blocking activity, ganglion blocking activity, and antagonism to the bradycardia induced by vagal stimulation were performed and the doses which produced an inhibition of 50% were calculated. Results in figures and tables are presented as mean $\pm$ standard error of the mean, when appropriate.

Drugs

Acetyl-β-methylcholine (Sigma), α-chloralose (British Drug Houses), pentobarbitone sodium solution (Abbott), pancuronium and analogues (Organon Scientific Development Group). All drugs except those which are monoquaternary, were dissolved in either 0·9% NaCl for experiments performed in vivo, or in Krebs–Henseleit (1932) solution for experiments performed in vitro. The monoquaternary compounds were dissolved (1 mg ml^{-1}) in citric acid (2·1 mg ml^{-1}) for purposes of stability. All concentrations of drugs are expressed in terms of mono or bis-quaternary salts.

RESULTS

Chick isolated biventer cervicis muscle preparation

All the compounds reduced responses to nerve stimulation without producing any contracture of the multiply innervated fibres of the preparation.

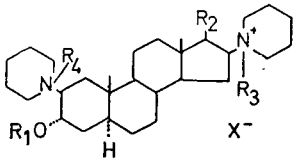
Anaesthetized cat

The 27 compounds tested comprise five groups according to their substitution at the 17β position on the 2β, 16β-dipiperidino-5α-androstane molecule; (i) 17β-acetates; (ii) 17β-propionates; (iii) 17β-butyrates; (iv) 17β-isobutyrates; (v) compounds without a 17-ester function. The molecular structures of the test compounds are included in Table 1.

The doses which produced a 50% reduction (calculated by regression analysis) of the twitch tension of the indirectly stimulated soleus and tibialis anterior muscles are shown in Table 1, with the respective onset and recovery times.

Tubocurarine is more potent in blocking the slow-contracting soleus muscle than the fast-contracting tibialis anterior muscle (Paton & Zaimis 1951). Most of the analogues of pancuronium were also slightly more potent on the soleus than on the tibialis anterior muscle, but eight compounds (9, 14, 15, 18,

Table 1. The neuromuscular blocking activities, vagal blocking activities and structures of I some 17β-acetoxy ammonio-androstanes, II 17β-propionyloxy ammonio-androstanes, III some 17β-butyryloxy ammonio-androstanes, IV 17β-isobutyryloxy ammonio-androstanes, V some 3α-hydroxy/acetoxya mmonio-androstanes. Compounds 1–6 were tested in four or more cats. Compounds 8, 12, 23 were tested in four cats. (Ac = acetyl, OAc = acetoxy, Bu = butyryl, OBu = butyryloxy, i-Bu = isobutyryl, O-i-Bu = isobutyryloxy, Et = ethyl, Me = methyl, Propg = propargyl, Pro = propionyl, OPro = propionyloxy.



	Compound	X^-	R_1	R_2	R_3	R_4	ED50 ($\mu g\ kg^{-1}$) Soleus/ tibialis	Onset (min) Soleus/ tibialis	Recovery (min) Soleus/ tibialis	ED50 ($\mu g\ kg^{-1}$) Vagal blockade	Vagal block/ Neuromuscular block (Soleus) Ratio
I	1	2Br	Ac	OAc	Me	Me	18/ 34	4·7 ± 0·4/ 4·3 ± 0·8	4·7 ± 0·7/ 8·8 ± 2·3	62	3·4
	2	Br	Ac	OAc	Me	—	34/ 38	3·8 ± 0·2/ 3·5 ± 0·6	2·9 ± 0·6 3·9 ± 0·5	2145	63·1
	3	2Br	H	OAc	Me	Me	56/ 59	2·3 ± 0·1/ 1·7 ± 0·1	6·8 ± 1·0/ 5·1 ± 1·2	263	4·7
	4	Br	H	OAc	Me	—	32/ 39	3·2 ± 0·3/ 2·8 ± 0·6	4·1 ± 0·2/ 4·1 ± 1·9	315	9·8
	5	Br	H	OAc	Et	—	12/ 17	4·5 ± 0·1/ 4·6 ± 1·5	3·9 ± 0·7/ 4·7 ± 1·0	280	23.3
	6	Br	H	OAc	Allyl	—	26/ 73	2·7 ± 0·2/ 2·5 ± 0·3	4·0 ± 0·6/ 6·0 ± 0·3	383	14·7
	7	Br	H	OAc	Propg	—	232/ —	1·3/ 1·0	—/ 2·2	528	2·3
II	8	2Br	Pro	OPro	Me	Me	82/ 87	2·5 ± 0·3/ 2·4 ± 0·3	5·1 ± 0·6/ 6·6 ± 1·7	564	6·9
	9	Br	Pro	OPro	Me	—	614/ 520	1·6/ 1·1	2·8/ 2·8	251	0·4
	10	2Br	H	OPro	Me	Me	496/ 544	1·4/ 1·3	2·8/ 2·5	296	0·6
	11	Br	H	OPro	Me	—	458/ 544	1·1/ 0·5	2·8/ 2·7	226	0·5
III	12	2Br	Bu	OBu	Me	Me	41/ 50	2·6 ± 0·5/ 2·0 ± 0·3	2·9 ± 0·6/ 4·3 ± 0·8	203	5
	13	Br	Bu	OBu	Me	—	391/ 405	1·5/ 1·5	3·7/ 3·6	265	0·7
	14	2Br	H	OBu	Me	Me	290/ 250	1·5/ 1·4	1·9/ 1·4	155	0·5
	15	Br	H	OBu	Et	—	270/ 260	1·7/ 1·3	4·4/ 3·5	215	0·8
	16	Br	H	OBu	Allyl	—	650/ 670	2·4/ 1·7	2·4/ 2·1	480	0·7
	17	Br	H	OBu	Propg	—	710/ 790	1·9/ 1·7	2·5/ 2·4	376	0·5
IV	18	2Br	i-Bu	O-i-Bu	Me	Me	165/ 161	1·4/ 0·9	3·6/ 1·9	328	2·0
	19	Br	i-Bu	O-i-Bu	Me	—	2000/ 2000	—	—	347	0·17
	20	2Br	H	O-i-Bu	Me	Me	436/ 511	2·9/ 2·6	4·9/ 3·1	220	0·5
V	21	2Br	Ac	OH	Me	Me	380/ 230	1·5/ 1·5	4·7/ 4·7	250	0·7
	22	2I	Ac	O	Me	Me	300/ 240	1·2/ 1·1	1·7/ 3·8	230	0·8
	23	2Br	Ac	H	Me	Me	128/ 121	1·9 ± 0·2/ 1·6 ± 0·1	2·4 ± 0·4/ 3·7 ± 0·4	125	1·0
	24	Br	H	OH	Me	—	2700/ 1850	3·8/ 1·4	1·7/ 3·0	250	0·1
	25	Br	Ac	O	Me	—	850/ 1100	1·4/ 1·4	4·7/ 5·1	400	0·5
	26	2I	H	O	Me	Me	1500/ 1650	2·5/ 3·5	—/ —	280	0·2
	27	I	H	O	Me	—	1380/ 2200	1·3/ 1·0	—/ 4·4	620	0·4

21, 22, 23 and 24) preferentially blocked the tibialis anterior.

All the compounds tested antagonized the bradycardial response to vagus nerve stimulation. However, the ratio of the doses required to block the vagus and to block neuromuscular transmission respectively varied considerably amongst the compounds. Thus, the ratio between the doses producing 50% inhibition of the responses of the heart to vagal stimulation and of the twitch response of the soleus muscle respectively was only 3·4 for pancuronium (1) but was over 60 for Org NC 45 (2). The 17β-acetates, of the five different series of compounds tested, had the greatest selectivity for the neuromuscular junction relative to the cardiac vagus.

The most potent of the neuromuscular blocking agents (1–6, 8, 12 and 23) also reduced the bradycardia produced by acetyl-β-methylcholine without inhibiting its depressor action in the same doses that blocked the responses to stimulating the cardiac vagus.

None of the compounds (1–6, 8, 12 and 23) tested for sympathetic ganglion blocking activity was active at neuromuscular blocking doses. However, at doses many times greater than those producing complete neuromuscular blockade, all of these potent neuromuscular blocking compounds depressed the contractions of the pre-ganglionically stimulated nictitating membrane in a dose-dependent manner. The doses of these neuromuscular blocking agents which produced 50% inhibition (calculated by regression analysis) of the pre-ganglionically stimulated nictitating membrane are shown in Table 2. Org NC 45 (2) possessed the least ganglion blocking activity of the compounds tested. Doses of 10 mg kg^{-1} Org NC 45 produced only 21 $\pm$ 3% block and it was estimated by extrapolation of the dose-inhibition curve that around 18 mg kg^{-1} would be required to reduce the responses of the pre-ganglionically stimulated nictitating membrane by 50%.

Table 2. The ED50 values for inhibition of the pre-ganglionically stimulated nictitating membrane determined by regression analysis († by interpolation * by extrapolation).

Compound	ED50 (mg kg^{-1})
17β-Acetates	
1.	4·3
2.	18·1*
3.	7·1
4.	6·8
5.	2·3
6.	8·0
17β-Propionate	
8.	0·9†
17β-Butyrate	
12.	2·2
3α-Acetate	
23.	8·0

Only one compound (6), of those tested (1–6, 8, 12 and 23) produced a slight (15·7 $\pm$ 0·4%, n = 4) depression of the responses of the post-ganglionically stimulated nictitating membrane at a dose of 5 mg kg^{-1}.

At neuromuscular blocking doses, only transient changes in blood pressure and heart rate were seen immediately after injection. At much higher doses, in the range that produced ganglion blockade (1 to 10 mg kg^{-1}) all the most potent neuromuscular blocking compounds tested (1–6, 8, 12 and 23) produced changes in blood pressure and all, except compound Org NC 45 (2), produced changes in heart rate. The changes produced are indicated in Table 3.

DISCUSSION

Two of the main cardiovascular side effects of the neuromuscular blocking agents currently in use are associated with autonomic blocking actions of the compounds. Tubocurarine and fazadinium both possess ganglion blocking activity (Randall 1951; Marshall 1973) which can cause hypotension. Gallamine, pancuronium, fazadinium and alcuronium block the cardiac muscarinic receptors (Riker & Wescoe 1951; Saxena & Bonta 1970; Marshall 1973; Hughes & Chapple 1976) which may contribute to an increase in heart rate. Thus, one goal in the attempts to produce improved neuromuscular blocking agents is to identify the molecular features required to produce neuromuscular blockade selectively.

All the compounds tested in this study reduced the twitch responses of the indirectly stimulated chick isolated biventer cervicis muscle without a concomitant contracture. This suggests that the compounds possess non-depolarizing neuromuscular blocking actions (Ginsborg & Warriner 1960) as would be expected from these bulky molecules. Only one of the most potent neuromuscular blocking agents tested (6) showed any post-ganglionic sympathetic blocking activity. The other compounds possessed sympathetic ganglion blocking activity. However, relative to the neuromuscular blocking potency, the sympathetic ganglion blocking potency of the compounds tested was very low and in fact can be disregarded. All the compounds tested antagonized the bradycardial response to vagal stimulation.

Table 3. Effects of pancuronium and analogues on blood pressure and heart rate (mean ±s.e. n = 4).

Compound	Dose (mg kg^{-1})	% Δ blood pressure	% Δ heart rate	Compound	Dose (mg kg^{-1})	% Δ blood pressure	% Δ heart rate
1	3	+37 ± 5	−2 ± 3	6	3	−11 ± 18	−10 ± 6·6
	5	+20 ± 29	+6 ± 4		5	−24 ± 23	−20 ± 17
	10	+6 ± 17	+1 ± 10		10	+13 ± 30	−5 ± 6
2	3	+42 ± 24	0	8	3	−40 ± 6	−31 ± 4
	5	+1 ± 28	0		5	−43 ± 7	−20 ± 5
	10	−28 ± 14	0		10	−31 ± 15	−16 ± 7
3	3	+34 ± 18	−1 ± 6	12	1	+19 ± 10	+1 ± 4
	5	+40 ± 24	−2 ± 8		3	+39 ± 12	+11 ± 3
	10	+54 ± 30	−26 ± 17		5	+27 ± 28	+33 ± 6
4	3	+24 ± 10	−2 ± 4	23	5	+143 ± 6	+11 ± 7
	5	+44 ± 8	−2 ± 6		10	+176 ± 15	+10 ± 10
	10	+94 ± 11	+3 ± 5		15	+191 ± 63	+46 ± 14
5	1	+24 ± 20	+7 ± 8				
	3	+1 ± 20	0				
	5	−11 ± 28	+1 ± 1				

The log-dose inhibition curves of the different compounds were similar in slope which probably indicates a common mode of action. The most potent neuromuscular blocking compounds reduced the bradycardia to both vagal stimulation and to acetyl-β-methylcholine in the same dose range. As the compounds did not reduce the depressor effect of acetyl-β-methylcholine, it is probable that, like pancuronium (Saxena & Bonta 1970), they possess an antimuscarinic action which is cardio-selective.

Several conclusions can be tentatively drawn from a study of structure-activity relationships within the five chemical series of compounds tested, with particular reference to blockade of neuromuscular transmission and of the cardiac muscarinic receptors.

It is clear from the present results that the 17β-functional group is an important determinant of neuromuscular blocking potency. Changing from a 17β-ester gives compounds (21–27) with relatively low neuromuscular blocking potency. Of the 17β-esters, the acetates as a series were the most potent neuromuscular blocking compounds tested in this study. This observation confirms that retention of an acetylcholine-like fragment in the D-ring results in high neuromuscular blocking potency (Savage et al 1971; Buckett et al 1973).

The *N*-alkyl substituent can influence the neuromuscular blocking potency in the 17β-acetoxy series; potency was retained with 16*N*-methyl, -ethyl and -allyl substituents but not with a propargyl group.

The importance of the acetylcholine-like fragment in the D-ring in the 17β-acetoxy series is most convincingly demonstrated by the fact that the change from a compound which has two quaternary nitrogen atoms at positions 2 and 16 (1 and 3) to the analogues with a tertiary nitrogen atom at position 2 and a quaternary nitrogen atom at position 16 (2 and 4) does not cause a marked reduction in neuromuscular blocking potency. In contrast, the analogous change from bis-quaternary to mono-quaternary in the 17β-propionyloxy, 17β-butyryloxy and 17β-isobutyryloxy series (compounds 8, 12 and 18, to 9, 13, and 19 respectively) is accompanied by a marked decrease in neuromuscular blocking potency. This suggests that although the D-ring fragment of the molecule is closely involved in determining the neuromuscular blocking potency of the 17β-acetates the same is not true for the less potent series. Further, in these less potent series (17β-propionates, 17β-butyrates and 17β-isobutyrates) the change from bis-quaternary 3α, 17β-diester (8, 12 and 18) to bis-quaternary 3α-hydroxyl-17β-monoester (10, 14 and 20) is also accompanied by a marked decrease in neuromuscular blocking potency. Thus, it seems that when the affinity of the D-ring fragment for the neuromuscular receptor is intrinsically weak, the structure of the A-ring fragment assumes a more important role in determining potency.

Sympathetic ganglion blocking potency was not associated with an intact acetylcholine-like fragment in either of the A or D-rings of the androstane nucleus. The low ganglion blocking potency of the compounds tested probably indicates that the interonium distance of the compounds is too long for potent ganglion blocking activity (Marshall & Martin-Smith 1972).

All the compounds containing an acetylcholine-like fragment in the A-ring (1, 22, 23) were amongst

the most potent of the compounds tested in blocking the cardiac muscarinic receptors. Further, the least potent compound in this respect (2) lacks an intact acetylcholine-like fragment in the A-ring. It is known that the acetylcholine-like fragment in the D-ring has a significantly different molecular geometry and electronic structure from that in the A-ring (Savage et al 1971). This strongly suggests that the conformation of the D-ring acetylcholine-like fragment, whilst intrinsically suited to the neuromuscular receptor, is relatively incompatible with the cardiac muscarinic receptor, whereas the isomeric fragment in the A-ring confers some affinity for this receptor.

From the present study, Org NC 45 (2) emerged as a compound of particular interest. This compound (2), a monoquaternary analogue of pancuronium, possessed high neuromuscular blocking potency, but very low potency in blocking the cardiac muscarinic receptors. Furthermore, the high selectivity of Org NC 45 (2) for blockade of neuromuscular transmission promises to satisfy one of the goals in the quest for new, improved neuromuscular blocking agents, and as such warrants further study.

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