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THE SPECIFICITY OF SOME AGONISTS AND ANTAGONISTS FOR NICOTINE-SENSITIVE RECEPTORS IN GANGLIA

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1 The guinea-pig isolated ileum has been used to estimate the ability of substituted phenylalkylonium salts (related to nicotine) to stimulate or block receptors in ganglia. The effects of hexamethonium were used to indicate which were the most specific ganglion stimulants; these were tested on the blood-pressure of pithed rats and for neuromuscular blocking activity on the rat diaphragm preparation.

2 *m*-Hydroxyphenylpropyltrimethylammonium and 3,4-dihydroxyphenethyltrimethylammonium (coryneine, 'quaternary dopamine') were the most active and specific ganglion stimulants but their usefulness *in vivo* may be limited by their neuromuscular blocking activity. The analogous tertiary compounds are being investigated.

3 The affinities of substances which were blocking agents at ganglionic receptors were measured on the isolated ileum with *m*-hydroxyphenylpropyltrimethylammonium as agonist. The affinities of selected compounds for postganglionic receptors were measured in experiments on the ileum in the presence of hexamethonium and with carbachol as agonist. Some of the compounds were tested for neuromuscular blocking activity on the rat diaphragm.

4 Phenylbutyldiethylamine had ganglion-blocking activity greater than pempidine and little postganglionic blocking or neuromuscular blocking activity. Its triethylammonium analogue had higher ganglion-blocking activity but had appreciable neuromuscular blocking activity.

5 The aromatic ring system is not essential either for activity or affinity and the effects of substituents are not related to their effects on electron distribution. Stimulant activity is enhanced only by hydroxyl or amino groups in suitable positions; it is not improved by the presence of rigid features (double or triple bonds or a cyclopropane ring) in the side chain. Affinity is slightly increased by chloro or bromo groups in suitable positions but the unsubstituted compounds are among those with the highest affinity. Substituents have similar effects on affinity for postganglionic receptors, though for these receptors the compounds mostly have only about one-tenth of their affinity for ganglionic receptors.

Introduction

Barlow, Thompson & Scott (1969) studied the effects of many compounds related to nicotine on the isolated rectus abdominis preparation of the frog. Compounds were found with 50 times the stimulant activity of nicotine; others had considerable blocking activity with values of log affinity constant as high as 5.9.

The present paper describes the properties of some of the most interesting of these compounds at other sites containing nicotine-sensitive receptors, particularly in parasympathetic ganglia. In addition some new compounds have been prepared in attempts to discover substances with high and specific ganglion-stimulant activity and others with high and specific ganglion-blocking activity. These included compounds with substituents not previously examined (fluoro and methyl), a few 3,4-dimethoxy- and 3,4-dihydroxy- compounds, and some unsubstituted compounds with unsaturated or cyclic (and therefore relatively rigid) side-chains. The high nicotine-like activity of 3,4-dihydroxyphenethyltrimethylammonium (the quaternary derivative of dopamine) has already been reported by Cuthbert (1964); this compound is also called coryneine and occurs in the cactus *Stetsonia coryne* (Reti, Arnolt & Luduena, 1935).

The activity and specificity of ganglion-stimulants was tested on the guinea-pig isolated ileum by assessing activity relative to a standard known to be reasonably specific and repeating the assessment in the presence of hexamethonium. Selected compounds were then tested separately

with a range of concentrations of hexamethonium, in order to see how far the dose-ratios obtained were consistent with competition. Compounds with ganglion-stimulant activity were also tested on the blood-pressure of pithed rats.

The most specific ganglion-stimulant was then used as an agonist in order to assess the affinity of antagonists for ganglionic receptors in the guinea-pig ileum. The extent to which the results were consistent with competition gave some indication of the specificity of the block. A rough idea of the neuromuscular blocking activity of many of the compounds was obtained by tests with the rat diaphragm preparation. The affinity constants of some of the compounds for postganglionic receptors in the ileum were also measured.

Methods

Guinea-pig ileum

The guinea-pig ileum was set up in aerated Tyrode solution at 37°C; responses were recorded isotonically with a load of about 0.5 gram. The interval between doses was 5 min, as described by Barlow & Franks (1971), to avoid desensitization. The agonist was in contact with the tissue for 30s and the drug solutions were applied by automated apparatus (Abramson, Barlow, Mustafa & Stephenson, 1969; Edinburgh Staff, 1970).

Agonists In the first group of experiments the activity of agonists was assessed relative to a standard, *p*-aminophenethyltrimethylammonium. Groups of responses were obtained with two concentrations of the standard, then with two concentrations of the test drug, and then again with two concentrations of the standard. The concentrations producing comparable responses were calculated from the average responses and the activity was expressed as the equipotent molar ratio, i.e.

$$\frac{\text{concentration of test}}{\text{concentration of standard}}$$

Mean values of the log ratio were calculated ± the s.e. mean.

In the second group of experiments the comparison with the standard was performed in exactly the same way but the Tyrode solution contained hexamethonium (3×10^{-5}M). From the estimate of the affinity constant of hexamethonium obtained by Barlow & Franks (1971), this should produce a dose-ratio of 8.8 but if the agonists are all acting at the same receptors as those blocked by hexamethonium, this should not affect their relative activities.

Some of the agonists were tested further by measuring the dose-ratios produced by various concentrations of hexamethonium. Responses were obtained with two concentrations of the agonist in the absence of hexamethonium and then with increased concentrations of agonist in the presence of hexamethonium. Usually two or three concentrations of hexamethonium were tested in any one experiment but in the present work the dose-ratio was calculated using only the responses obtained in the absence of hexamethonium at the start of the experiment. In the work of Barlow & Franks (1971) responses were also obtained in the absence of hexamethonium after two concentrations of hexamethonium had been tested but these were quite often found to differ appreciably from those obtained at the start of the experiment; it is probably more satisfactory to test a smaller number of concentrations of hexamethonium in any one experiment and to perform experiments with more pieces of ileum.

Antagonists The antagonism produced by compounds was assessed by their effects on the responses to *m*-hydroxyphenylpropyltrimethylammonium, which seemed to be the most suitable agonist (see results). Each antagonist was usually tested in two concentrations, producing dose-ratios of about 10 and about 20, and the exact dose-ratio and concentration of antagonist were used to calculate the affinity constant. Results were expressed as the mean value of log affinity constant ± the s.e. Some compounds did not act competitively and could be tested only in one concentration because the antagonism was unsurmountable. This could be due to a postganglionic blocking action (i.e. atropine-like activity) and only a rough idea of their effects at ganglionic receptors could be obtained.

Postganglionic receptors The affinity constants of selected compounds for postganglionic receptors in the ileum were measured in experiments with carbachol as agonist, allowed to act for 30s and given once every 90s, and with hexamethonium, 2.76×10^{-4}M, present in the Tyrode solution (Barlow, Scott & Stephenson, 1963; Edinburgh Staff, 1970; Barlow, Franks & Pearson, 1973). The selection was intended to show the effects of chain length and of type of substituent on affinity, for comparison with their effects on affinity for receptors in ganglia. Some compounds were also included for which the results in the experiments on ganglionic receptors (see above) suggested that they should also be acting postganglionically.

Effects on blood-pressure

Pithed male Wistar rats, weighing between 250 and

500 g, were anaesthetized with ether; the trachea was cannulated, and a 14 gauge needle passed through the eye and down the spinal cord. Ventilation was maintained artificially with air from a Palmer pump, at a rate of 1 stroke/s and with a stroke volume of 5 ml. The animal was kept on a heated operating table and the left external jugular vein was cannulated with a nylon catheter (0.63 mm o.d.), which was used for administering drugs. The left carotid artery was cannulated with a nylon catheter (0.75 mm o.d.) which was connected to a Consolidated blood-pressure transducer attached to a Devices M2 hot-stylus recorder. Heparin (200 units) and atropine sulphate (0.4 mg; 1.14 mmol) were administered and the mean blood-pressure was recorded continuously.

Drugs were given by infusion through the vein cannula with a Watson Marlow MRHE pump in a period of 15 seconds. Different doses were given by altering the rate of infusion and the interval between doses was 4 to 5 minutes. The activity of compounds was expressed relative to that of a standard, *p*-aminophenethyltrimethylammonium, by comparing doses producing comparable rises in pressure. The standard was tested twice at three dose-levels during each experiment and it was usually possible to compare two or three compounds with it in any one animal also at three dose-levels tested twice. The rise in blood-pressure observed was biphasic. The second rise was absent after adrenalectomy and the first rise was blocked by bretylium. Both these effects were expressed as the rise in pressure divided by the blood-pressure before the drug was given (which did not vary much during the experiment) and the values were used to calculate log dose-response lines by the method of least squares. Equipotent molar ratios were calculated from the ratios of the doses producing responses which were 20% and 80% of the maximum observed. With the particular compounds tested, these equipotent molar ratios for the two phases in the response were approximately the same so the results could be expressed as a single ratio.

Rat diaphragm

The rat isolated diaphragm preparation was set up as described by Bülbring (1946) but in Krebs solution instead of Tyrode (Edinburgh Staff, 1970). Responses were recorded isometrically with a Devices strain-gauge and recorder. The phrenic nerve was stimulated once every 10s by rectangular pulses of about 0.5 ms duration, at a voltage (usually about 2V) which produced maximal twitch responses. Drugs were added by hand once every 15 min and allowed to act for 5 minutes. In each experiment compounds were tested in doses of 0.02 and 0.04 ml of 10^{-1}M solutions and 0.10 ml of 10^{-2}M; the bath volume was approximately 20 ml. The percentage inhibition of the contractions was calculated and the total ('score') for the three doses was taken. Each compound was tested on two preparations and the average score used as a very rough estimate of relative neuromuscular blocking activity.

Compounds

The new compounds are listed in Table 1. The other compounds tested have been described by Barlow, *et al.* (1969). Pempidine tartrate, included in the tests of ganglion-blocking activity, was obtained from May & Baker; nicotine hydrogen tartrate from BDH Ltd; dimethylphenylpiperazinium iodide (DMPP) from Aldrich.

The synthesis of some other new substituted phenylpropyl compounds used has been described elsewhere (Ison & Hassan, 1973).

Trans-2-phenylcyclopropyltrimethylammonium was prepared from the amine (Burger & Yost, 1948; Smejkal & Farkas, 1963); phenylprop-1-ynyltrimethylammonium was prepared from the dimethylamino compound (Iwai & Hiraoka, 1963), which was also partially hydrogenated using Lindlar catalyst and a drop of quinoline to the *cis* form of 3-dimethylamino-1-phenylprop-1-ene and quaternized. *Trans*-3-dimethylamino-1-phenylprop-1-ene was prepared by dehydration of the alcohol (Ison & Casy, 1971); the assignment of configuration was supported by the intervinylic coupling constants (J= 16 Hz for the *trans*-dimethylamino compound; J= 8 Hz for the *cis*-isomer; both appeared to be isomerically pure).

Difficulties were experienced in the repetition of the synthesis of leptodactyline (*m*-hydroxyphenethyltrimethylammonium). Samples were obtained which had m.p. 251-2°C (cf. 163-4°C) and had low biological activity even though the analysis was acceptable for leptodactyline bromide (found Br^-, 30.94; theory, 30.70%). the n.m.r. spectrum however, indicated that this, and the methoxycompound from which it was derived, were tetrahydro*iso*quinoline derivatives, formed by Pictet-Spengler cyclisation during the methylation of *m*-methoxyphenethylamine. 6-Methoxy-2-methyl-1,2,3,4-tetrahydro*iso*quinoline methiodide had m.p. 170-173°C (found I^-, 39.77, theory, 39.75, cf. *m*-methoxyphenethyltrimethylammonium iodide, m.p. 182.3-182.6°C, theory, I^- 39.51). The theoretical content of bromide for 6-hydroxy-2-methyl-1,2,3,4-tetrahydro*iso*quinoline methobromide is 30.94, identical with that found. *m*-Methoxyphenethyldimethylamine was therefore prepared from the reduction of the dimethylamide (Epstein, Plapinger, Michel, Cable,

Stephani, Hester, Billington & List, 1964) and the methobromide had m.p. 163-4°C.

Results

Ganglion stimulants

The relative activity of the compounds on the guinea-pig ileum is shown in Table 2. Several of them were more active than the standard, *p*-aminophenethyltrimethylammonium, but some of these were even more active in the presence of hexamethonium (with positive values of Δ in Table 2) indicating that they were acting, at least in part, at postganglionic receptors (or ganglionic receptors not blocked by hexamethonium). This was most marked with the β-pyridylmethyl, phenethyl, cyclopentylpropyl, phenylcyclopropyl, phenylpropynyl compounds and leptodactyline. With

Table 1 Melting points and analyses of new compounds.

$R'C_6H_4CH_2\overset{+}{N}Me_3\ X^-$					
R'=		*X*⁻=	*m.p.*	*Found (X⁻)*	*Theory*
o-Me		Br^-	206-8	32.62	32.72
m-Me		Br^-	218-9	32.58	32.72
p-Me		I^-	213	43.73	43.58
p-F		I^-	248	42.71	42.99
$R''R'C_6H_3CH_2CH_2\overset{+}{N}Me_3\ X^-$					
R''=	*R'*=				
m-F	H	I^-	232-3	40.85	41.04
p-F	H	I^-	251-4	40.86	41.04
p-MeO	*m*-MeO	I^-	230	36.00	36.14
p-HO	*m*-HO	I^-	207-9*	C, 41.1	40.9
				H, 5.46	5.61
$R''R'C_6H_3CH_2CH_2\overset{+}{N}Et_2H\ X^-$					
p-Br	H	Cl^-	142	12.16	12.12
p-MeO	*m*-MeO	Cl^-	126-7	13.19	12.95
$R''R'C_6H_3(CH_2)_3\overset{+}{N}Me_3X^-$					
p-Me	H	I^-	119-120	39.45	39.76
p-MeO	*m*-MeO	I^-	133	34.68	34.75
p-HO	*m*-HO	Br^-	200-201	C, 49.7	49.7
				H, 7.08	6.95
$BrC_6H_4CH{=}CHCH_2\overset{+}{N}Et_2H$	*trans*	Cl^-	161	11.69	11.65
$C_6H_5C{\equiv}CCH_2\overset{+}{N}Me_3\ I^-$			226-8	42.01	42.13
$C_6H_5CH{=}CHCH_2\overset{+}{N}Me_3\ I^-$	*cis*		116-7	42.16	41.86
	trans		182-3	42.02	41.86
$C_6H_5\Delta\overset{+}{N}Me_3\ I^-$	*trans*		182-4	42.23	41.86
$C_6H_5(CH_2)_4\overset{+}{N}Et_2H$		Br^-	99-100	27.85	27.90
$C_6H_5(CH_2)_5\overset{+}{N}Et_2H$		Br^-	79	26.62	26.61
$C_5H_9(CH_2)_3\overset{+}{N}Me_3$		I^-	208	42.72	42.70
$C_5H_9(CH_2)_3\overset{+}{N}Et_2H$		Br^-	95-97	30.32	30.22
$C_6H_{11}(CH_2)_3\overset{+}{N}Me_3$		I^-	173	40.56	40.78
$C_6H_{11}(CH_2)_3\overset{+}{N}Et_2H$		Br^-	118-9	28.72	28.72

Melting points were measured with a Mettler FP1 instrument, usually at a rate of heating of 2°C/min, and are uncorrected. Analyses for halide are gravimetric (with samples of 50-250mg) and for C and H are micro- by Drs Weiler and Strauss, Oxford. The quaternary derivative of dopamine, marked with an asterisk, was prepared by Barger & Ewins (1911), who recorded m.p. 205°C and m.p. 232°C for the dimethoxy compound. Details of other compounds studied are given by Barlow, *et al.*, (1969) and Ison & Hassan (1973).

some others hexamethonium reduced activity relative to *p*-aminophenethyltrimethylammonium. In most instances this indicated that the compounds had postganglionic blocking activity and this was investigated in experiments with carbachol as agonist (see below). The variation in the effects of hexamethonium on the actions of the phenylpropyl compound (Table 2) could be ascribed to a lower sensitivity of the receptors in ganglia in the second group of experiments with the consequent need to use higher concentrations which produced detectable postganglionic blocking effects.

The compounds which were considered to be most interesting were tested in the presence of various concentrations of hexamethonium in order to see to what extent the antagonism appeared to

Table 2 Stimulant activity on the isolated guinea-pig ileum.

	Hexamethonium concentration		
	0	*3 x 10⁻⁵ M*	Δ
β-pyridylCH$_2$N$^+$Me$_3$	$\bar{1}$.25 ± 0.06 (5)	$\bar{2}$.73 ± 0.06 (3)	+0.52
β-pyridylCH$_2$CH$_2$N$^+$Me$_3$	$\bar{1}$.52 ± 0.02 (4)	$\bar{1}$.35 ± 0.01 (2)	+0.17
m-HOC$_6$H$_4$(CH$_2$)$_3$NMe$_3$	$\bar{1}$.69 ± 0.02 (5)		
pm-(HO)$_2$C$_6$H$_3$CH$_2$CH$_2$N$^+$Me$_3$ (coryneine)	$\bar{1}$.82 ± 0.01 (4)	$\bar{1}$.68 ± 0.02 (3)	+0.14
m-HOC$_6$H$_4$CH$_2$CH$_2$N$^+$Me$_3$ (leptodactyline)	$\bar{1}$.92 ± 0.02 (5)	$\bar{1}$.07 ± 0.02 (4)	+0.85
p-H$_2$NC$_6$H$_4$CH$_2$CH$_2$N$^+$Me$_3$	0.0 (standard)	0.0	
C$_6$H$_5$CH=CHCH$_2$N$^+$Me$_3$ *trans*	0.07 ± 0.01 (4)	0.47 ± 0.04 (3)	−0.40
Ph(CH$_2$)$_2$N$^+$Me$_3$	0.20 ± 0.02 (3)	$\bar{1}$.4	+0.8
pm-(HO)$_2$C$_6$H$_3$(CH$_2$)$_3$N$^+$Me$_3$	0.42 ± 0.03 (6)	0.35 ± 0.02 (3)	+0.07
C$_6$H$_5$(CH$_2$)$_3$N$^+$Me$_3$ (1) (2)	0.60 ± 0.04 (5) 0.61 ± 0.01 (3)	0.68 ± 0.01 (3) 0.91 ± 0.1 (2)	−0.08 −0.3
p-HOC$_6$H$_4$CH$_2$CH$_2$N$^+$Me$_3$ (hordenine methiodide)	0.79 ± 0.04 (4)		
C$_5$H$_9$(CH$_2$)$_3$N$^+$Me$_3$	0.84 ± 0.02 (3)	0 approx.	+0.8
p-HOC$_6$H$_4$(CH$_2$)$_3$N$^+$Me$_3$	0.86 ± 0.03 (4)		
PhenylcyclopropylN$^+$Me$_3$ *trans*	0.90 ± 0.06 (3)	0 approx.	+0.8
m-H$_2$NC$_6$H$_4$CH=CHCH$_2$N$^+$Me$_3$ *trans*	0.91 ± 0.01 (4)		
p-H$_2$NC$_6$H$_4$(CH$_2$)$_3$N$^+$Me$_3$	1.03 ± 0.01 (4)		
C$_6$H$_5$C≡CCH$_2$N$^+$Me$_3$	1.12 ± 0.08 (3)	0.3 approx.	+0.8
2-methyl-7-hydroxytetrahydroisoquinoline methiodide*	1.60 ± 0.06 (2)		

The numbers show the mean estimate of the logarithm of the equipotent molar ratio relative to *p*-aminophenethyltrimethylammonium ± s.e., with the number of estimates in parentheses. Note that a negative number indicates greater activity because the same responses are produced with smaller concentrations. The column marked Δ shows the effect of hexamethonium: if the compound is as specific as the standard the value should be zero. A positive value suggests postganglionic stimulant (muscarine-like) activity; a negative value suggests postganglionic blocking (atropine-like) activity. The phenylpropyl compound was tested in two separate sets of experiments (1) and (2), and the effects of hexamethonium were found to be variable, though the relative activity in the absence of hexamethonium was the same. The compound marked with an asterisk was found accidentally in the synthesis of leptodactyline (see above) and is only very feebly active.

m-F$_3$CC$_6$H$_4$(CH$_2$)$_3$N$^+$Me$_3$, C$_6$H$_5$(CH$_2$)$_4$N$^+$Me$_3$ and C$_6$H$_{11}$(CH$_2$)$_3$N$^+$Me$_3$ appeared to be inactive but the two latter blocked the actions of carbachol. C$_5$H$_9$(CH$_2$)$_3$NMe$_3$ appeared to be a partial agonist at postganglionic receptors with log K about 4.2.

be competitive; the results are shown in Table 3. These agree reasonably with results already obtained for two of the compounds by Barlow & Franks (1971). There are differences, which suggest that the errors may be bigger than the variance indicates, but these could partly be due to differences in experimental technique (discussed in the methods section). With the lower concentrations of hexamethonium there is good agreement between the experimental dose-ratios for most of the compounds and those calculated assuming a value of 2.6×10^5 for the affinity constant of hexamethonium.

The low dose-ratios obtained with β-pyridylethyltrimethylammonium show that it acts to a large extent at receptors not blocked by hexamethonium and the high dose-ratios obtained with phenylpropyltrimethylammonium indicate that it has appreciable postganglionic blocking (atropine-like) activity, though this is not as much as that of dimethylphenylpiperazinium (DMPP), for which Barlow & Franks (1971) obtained dose-ratios of 12 with only 2×10^{-5}M hexamethonium.

From the results obtained with the highest concentration of hexamethonium, 1.6×10^{-4}M, it seemed that *m*-hydroxyphenylpropyltrimethylammonium was the most specific in that the results were closest to those consistent with competition. It was therefore chosen as the agonist for testing ganglion-blocking activity with the preparation. The results obtained with *p*-aminophenethyltrimethylammonium were not as consistent with competition as those previously obtained (Barlow & Franks, 1971), but these did not include experiments with 1.6×10^{-4}M hexamethonium. The results confirm the high nicotine-like activity of the quaternary derivative of dopamine (coryneine), reported by Cuthbert (1964), but the compound is more difficult to make than the *p*-aminophenethyl- and *m*-hydroxyphenylpropyl- compounds and slowly oxidizes in solution, turning pink, so it was considered less suitable for extensive use as a ganglion-stimulant. It was, however, slightly more active than these compounds on the rat blood-pressure (Table 4).

Most of the ganglion-stimulants were also potent blocking agents on the rat diaphragm (Table 5) so their potential usefulness *in vivo* might be limited by their neuromuscular blocking properties. It was not possible to assess this from the experiments on pithed rats because these were artificially ventilated, but the lowest concentration

Table 3 Dose-ratios produced by hexamethonium with different agonists on the guinea-pig ileum.

	Hexamethonium concentration ($\times 10^{-5}$ M)				*Agonist concentration*
	2	*4*	*8*	*16*	
Calculated dose-ratio	6.2	11.4	21.8	42.5	
p-$H_2NC_6H_4CH_2CH_2\overset{+}{N}Me_3$	7.2*	14.7*	21.0*		
	4.7 ±0.5 (2)	8.1 ±0.3 (2)	14.4 ±1.0 (7)	19.0 ±2.9 (2)	4×10^{-6} M
m-$HOC_6H_4(CH_2)_3\overset{+}{N}Me_3$	6.4*	10.3*	15.9*		$2.\times 10^{-6}$ M
			17.6 ±2.3 (3)	52.0 ±1.3 (2)	
pm-$(HO)_2C_6H_3CH_2CH_2\overset{+}{N}Me_3$		12.4 ±1.6 (2)	20.4 ±2.6 (3)	69.4 (1)	3×10^{-6} M
pm-$(HO)_2C_6H_3(CH_2)_3\overset{+}{N}Me_3$		11.6 ±0.4 (3)	18.0 ±1.5 (3)		7×10^{-6} M
$C_6H_5(CH_2)_3\overset{+}{N}Me_3$		16.7 ±2.1 (4)	38.0 (1)		1.1×10^{-5} M
β-pyridyl$CH_2CH_2\overset{+}{N}Me_3$		4.1 ±0.3 (3)	5.2 ±0.4 (3)	8.3 ±0.3 (3)	5×10^{-6}

Mean values are shown ± s.e., with the number of results in parentheses. Values marked with an asterisk were obtained by Barlow & Franks (1971).

used on the rat diaphragm was about 5×10^{-5}M, compared with concentrations of 2 or 3×10^{-6}M used to stimulate the guinea-pig ileum (Table 3). The results suggest then that *m*-hydroxyphenylpropyltrimethylammonium and coryneine should have considerable specificity for ganglia unless there are large differences in species sensitivity (which is possible with desensitizing neuromuscular blocking agents). In concentrations up to 3×10^{-5}M, *m*-hydroxyphenylpropyltrimethyl was without effect on guinea-pig strip preparations, free from parasympathetic ganglia, nor did it affect the responses to carbachol (Fiona Roberts, personal communication).

Ganglion-blocking agents

Estimates of log affinity constant with *m*-hydroxyphenylpropyltrimethylammonium as agonist are shown in Table 6. With several of the compounds it was only possible to obtain dose-ratios less than 10; with higher concentrations, the antagonism was unsurmountable, presumably because the compounds blocked the postganglionic receptors. A few compounds also caused the development of spontaneous activity in the resting ileum. The results in Table 6 also include estimates of relative neuromuscular blocking activity on the rat diaphragm. These are only very approximate, because

Table 4 Pressor activity in pithed rats.

$R-\overset{+}{N}Me_3$	*log mean epmr*	
R=		
pm-$(HO)_2C_6H_3CH_2CH_2CH_2$- (coryneine)	1.8	(5)
p-$H_2NC_6H_4CH_2CH_2$-	0 (standard)	
m-$HOC_6H_4CH_2CH_2$- (leptodactyline)	0.0	(6)
m-$HOC_6H_4CH_2CH_2CH_2$-	0.0	(11)
Suberylcholine	0.0	(5)
pm-$(HO)_2C_6H_3CH_2CH_2CH_2$-	0.1	(6)
β-pyridyl-CH_2-	0.1	(7)*
β-pyridyl-CH_2-CH_2-	0.15	(10)*
C_6H_5CH=CH-CH_2- (trans)	0.2	(4)
o-$H_2NC_6H_4CH_2CH_2$-	0.5	(9)
m-$HOC_6H_4CH_2$-	0.6	(9)
m-$H_2NC_6H_4CH_2CH_2$-	0.6	(10)
Dimethylphenylpiperazinium (DMPP)	0.7	(5)*
Nicotine	*	
Phenylcyclopropyl- (trans)	*	
m-$MeC_6H_4CH_2CH_2$	*	
m-$FC_6H_4CH_2CH_2$	*	
p-$H_2NC_6H_4CH_2CH_2CH_2$	*	
p-$H_2NC_6H_4CH$=$CHCH_2$ (trans)	*	
$C_6H_5C{\equiv}CCH_2$	*	

The standard stimulant was *p*-aminophenethyltrimethylammonium and the figures show the logarithm of the mean equipotent molar ratio (epmr) with the number of comparisons in parentheses. An asterisk indicates that stimulant activity was accompanied by desensitisation which in some instances made it impossible to obtain a quantitative result.

The following (trimethylammonium) compounds were blocking agents without themselves producing a rise in blood pressure:

p-$H_2NC_6H_5CH_2$-, *p*-$MeC_6H_4CH_2$-, *p*-$FC_6H_4CH_2$-,
o-$MeC_6H_4CH_2CH_2$-, *p*-$MeOC_6H_4CH_2CH_2$-,
pm-$(MeO)_2C_6H_3CH_2CH_2$-, *p*-$FC_6H_4CH_2CH_2$-,
p-$MeC_6H_4CH_2CH_2CH_2$-, *p*-$MeOC_6H_4CH_2CH_2CH_2$-,
pm-$(MeO)_2C_6H_3CH_2CH_2CH_2$-, β-pyridyl$CH_2CH_2CH_2$-,
C_6H_5CH=$CHCH_2$- (*cis*).

the result is greatly affected by the order in which the compounds are given. They do indicate, however, that although high ganglion-blocking activity is often associated with high neuromuscular blocking activity, with many chloro compounds for instance, though there are others, such as *p*-bromobenzyldimethylamine, *o*-methoxyphenylpropyltriethylammonium, phenylpropyl- and phenylbutyldiethylamine, where there appears to be appreciable specificity for ganglia.

Affinity constants for postganglionic receptors are shown in Table 7.

Ionization

Although some of the compounds studied were tertiary bases, these are likely to be largely ionized at body pH. Barlow, *et al.* (1969) recorded a pK_a of 9.14 for phenethyldiethylamine in 40% v/v ethanol at 25°C and we have obtained a value of 9.3 for this compound in pure water at 25°C. The corresponding values for the benzyl, phenylpropyl and phenylbutyl diethylamines were 9.1, 9.6 and 9.8, respectively.

Discussion

The results indicate the potential value of *m*-hydroxyphenylpropyltrimethylammonium and 3,4-dihydroxyphenethyltrimethylammonium (coryneine) as ganglion stimulants in *in vitro* tests. Further work is needed to see how far their usefulness *in vivo* may be limited by neuromuscular blocking activity and it seems worth testing their tertiary dimethylamino analogues to see if these retain ganglion-stimulant activity but lack neuromuscular blocking properties. The change from triethylammonium to diethylamino effectively restricts blocking activity to ganglia (see below) so it is possible that the same may occur with stimulants.

Table 5 Neuromuscular blocking activity of ganglion-stimulants on the isolated phrenic-nerve-diaphragm preparation.

(+)-Tubocurarine chloride	300
p-$H_2NC_6H_4CH_2CH_2\overset{+}{N}Me_3$	300
pm-$(HO)_2C_6H_3CH_2CH_2\overset{+}{N}Me_3$ (coryneine)	250
m-$HOC_6H_4CH_2CH_2CH_2\overset{+}{N}Me_3$	240
m-$HOC_6H_4CH_2CH_2\overset{+}{N}Me_3$ (leptodactyline)	230
Decamethonium iodide	230
$C_6H_{11}CH_2CH_2CH_2\overset{+}{N}Me_3$	180

The maximum score in the test is 300, indicating complete block in 5 min at all three dose-levels (0.04 and 0.02 ml of 10^{-1}M and 0.1 ml of 10^{-2}M added to a 20 ml bath). Each compound was tested on two preparations. Even though the interval between doses was at least 15 min, the effects of a dose depend greatly on those of previous doses and the error in the score is likely to be at least ± 50.

Table 6 Ganglion-blocking activity on guinea-pig ileum and neuromuscular blocking activity.

	X-C_6H_4-$(CH_2)_n$-R			
n= 1				
R= $\overset{+}{N}Et_2H$	o	m	p	H
X = Cl	5.57 (8)*	5.50 (4)	4.69 (1)*	5.25 (6)
	20	*100*	*25*	*0*
Br		5.67 (5)*	6.42 (4)	
		0	*75*	
OH			4.96 (5)	
			0	
R= $\overset{+}{N}Et_3$				
X = Cl		5.35 (5)	5.53 (2)	5.82 (6)
		5	*80*	*10*
Br			5.86 (7)	
			135	

Table 6 *continued*

$X\text{-}C_6H_4\text{-}(CH_2)_n\text{-}R$

	o	m	p	H
n= 2				
R= $\overset{+}{N}Et_2H$				
X= Cl	5.7**			5.64 (6) *0*
Br			5.64 (4)	
R= $\overset{+}{N}Et_3$				
X= Cl		6.15 (4) *220*	6.26 (4) *200*	5.99 (4) *45*
NO_2		4.70 (4) *15*		
n= 3				
R= $\overset{+}{N}Et_2H$				
X= Cl	6.11 (6) *60*	6.04 (4)* *90*	5.4** *80*	6.06 (5) *0*
R= $\overset{+}{N}Et_3$				
X= Cl		6.64 (8) *210*	6.30 (3) *190*	6.19 (8)* *130*
OH		5* *85*	4.33 (3)* *50*	
OMe	6.28 (5) *55*	5.96 (4)		
n= 4				
R= $\overset{+}{N}Et_2H$				6.27 (5) *10*
$\overset{+}{N}Et_3$				6.86 (5)* *140*
n= 5				
R= $\overset{+}{N}Et_2H$				5.68 (4)* *25*
$\overset{+}{N}Et_3$				7.03 (5)* *100*
$PhCH{=}CHCH_2NMe_3$ (cis)		4.11 (3)*		
$p\text{-}BrC_6H_4CH{=}CHCH_2NEt_2$ (trans)		5.81 (6)*		
$C_5H_9(CH_2)_3NEt_2$		5.52 (6)		
$C_6H_{11}(CH_2)_3NEt_2$		6.39 (3)*		
Pempidine		6.09 (5) *0*		
(+)-Tubocurarine chloride		5.55 (4) *300*		
Decamethonium iodide		*230*		

Mean estimates of log affinity constant are shown with the number of estimates in parentheses. The standard error was usually less than 0.1 log units. Results for compounds with which it was only possible to obtain dose-ratios less than 10 are marked with an asterisk; the others are based on dose-ratios of up to 20. Some compounds produced a marked disturbance of the ileum which resembled spontaneous activity and this is indicated by a double asterisk. The number in italics is the average score in the tests on the rat diaphragm (maximum = 300).

Relationships between structure and activity appear to be complex but this is partly because the results in the experiments on the ileum are complicated by actions at postganglionic receptors. The optimum chain length, for instance, is apparently two methylene groups in the *p*-amino- and 3:4-dihydroxy compounds but three methylene groups in the *m*-hydroxy- compounds. It is also apparently two methylene groups in the unsubstituted compounds, but this is partly due to postganglionic stimulant activity in the phenethyl compound. The further possible complication of postganglionic blocking activity makes it impossible to say whether ganglion-stimulant acitivity increases or decreases with side-chains longer than three methylene groups.

Substituents other than hydroxyl or amino markedly reduce activity and there is no reason to link activity with the electronic effects of substituents. The aromatic nature of the ring may not, in fact, be essential because the cyclopentyl compound has some stimulant activity, though the results are complicated by its weak postganglionic stimulant activity (it appears to be a partial agonist at postganglionic receptors). It has been suggested that activity might be enhanced by the presence of rigid features in the side chain (Wong & Long, 1962; Kirkendol, Woodbury & Elko, 1972) but our results do not support this. *cis*-Phenylpropenyltrimethylammonium is a weak blocking agent and although the *trans* isomer, and *trans*phenylcyclopropyltrimethylammonium have some activity (Table 2), this is partly postganglionic. The present results do not, however, rule out the suggestion (Barlow, *et al.* 1969) that activity 'is associated with the presence of substituents which can interact with water molecules which may be involved in the action of the drug at the receptor'.

With the ganglion-blocking agents it is possible to observe the effects of substituents on affinity for ganglionic and postganglionic receptors (Table 8) and it is remarkable how often the introduction of a substituent reduces affinity and how seldom it enhances it to any great extent. It is also striking that the effects of substituents on affinity are qualitatively similar even though the compounds

Table 7 Affinity for postganglionic receptors.

			$Ph(CH_2)_nNEt_2$		
n=	1	2	3	4	5
	3.818 (3)	4.253 (4)	4.809 (4)	4.980 (6)	5.595 (6)
			$X\text{-}C_6H_4\text{-}(CH_2)_3R$		
X=Cl			o	m	p
$R=\overset{+}{N}Et_2H$			4.894 (3)	5.13* (2)	4.75* (1)
$R=\overset{+}{N}Et_3$			5.51* (3)	5.427 (5)	5.243 (4)
			$m\text{-}XC_6H_4(CH_2)_n\overset{+}{N}Et_3$		
n=1					
X=Br			5.190 (4)		
H_2N			4.365 (4)		
n = 2					
X=Cl			5.179 (5)		
O_2N			4.430 (4)		
HO			4.357 (4)		
n = 3					
X=HO			5.081 (4)		
MeO			4.789 (4)		
$C_5H_9(CH_2)_3NEt_2$			4.785 (5)		
$C_6H_{11}(CH_2)_3NEt_2$			5.333 (5)		
(+)-Tubocurarine chloride			about 5.3 (3)		

Values of log affinity constant are shown with the number of estimates in parentheses. The standard error of the estimates was usually about 0.04 log units and never exceeded 0.10 log units. Compounds which disturbed the resting intestine are indicated by an asterisk. The value for (+)-tubocurarine chloride is only approximate; the compound did not appear to act competitively.

Table 8 Effects of changes in structure on affinity for ganglionic and postganglionic receptors.

$X\text{-}C_6H_4\text{-}(CH_2)_n\overset{+}{N}Et_3$

	logKg	*logKpg*	Δg	Δpg
n= 1				
X= H	5.25	4.83		
m– Br			+0.42	+0.36
m– H_2N				–0.47
n= 2				
X= H	5.99	4.95		
m– Cl			+0.16	+0.23
m–O_2N			–1.29	– 0.52
m–HO				–0.59
n= 3				
X= H	6.19	5.18		
m–HO			–1.2	–0.10
m–MeO			–0.23	–0.40

$X\text{-}(CH_2)_3R$

R = $\overset{+}{N}Et_2H$				
X = H	6.06	4.81		
o-Cl			+0.05	+0.08
m-Cl			–0.02	+0.32
p-Cl			–0.66	–0.06
R = $\overset{+}{N}Et_3$				
X = H	6.19	5.18		
o-Cl				+0.38
m-Cl			+0.45	+0.24
p-Cl			+0.11	+0.06

Values of log K are shown for the unsubstituted compounds and the columns marked Δ show the effect produced by the substituent. The subscripts g and pg refer to values for ganglionic and postganglionic receptors, respectively.

have usually about ten times the affinity for ganglionic receptors as they have for postganglionic ones (this can be seen from the distribution of + and − signs in Table 8). The affinity of the cyclopentyl and cyclohexyl compounds (Table 6 and 7) indicates that the aromatic ring is not essential for binding and there is no reason to suppose that the effects of a group on affinity are simply related to its effects on electron distribution. The effects of a substituent on affinity are different in the different series of compounds and the results support the view (Barlow, *et al.* 1969) that with these compounds affinity and substituent cannot be fitted into an equation such as those described by Hansch (Hansch, Maloney, Fujita & Muir, 1962; review by Tute, 1971).

The considerable specificity of the blocking agents for ganglionic receptors is illustrated for the unsubstituted compounds in Figure 1. Phenylbutyltriethylammonium has some neuromuscular blocking activity but this is much less in its analogous tertiary base, phenylbutyldiethylamine. This latter compound appears to be a ganglion-blocking agent with considerable activity and specificity, easy to make, and potentially capable of crossing membranes, such as those of the gastrointestinal tract or the 'blood-brain barrier'. Phenylpropyldiethylamine is a weaker ganglion-blocking agent that may be even more specific (Table 6). The properties of these compounds appear to be worth investigating in greater detail.

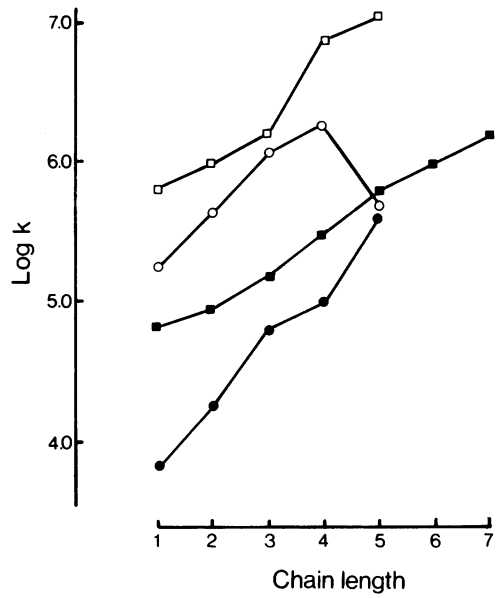

Fig. 1 Values of log K for postganglionic receptors (■,●) and for receptors in ganglia (□,○) are plotted against the number of methylene groups (chain length) for the compounds $Ph(CH_2)_n\overset{+}{N}Et_3$ (■ and □) and $Ph(CH_2)_n\overset{+}{N}Et_2H$ (● and ○). Note the greater affinity of both types of compound for ganglionic receptors and the considerable affinity of the tertiary bases, particularly of phenylbutyldiethylammonium (chain length = 4).

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