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EFFECTS OF SOME ISOMERS AND ANALOGUES OF NICOTINE ON JUNCTIONAL TRANSMISSION

BY

R. B. BARLOW AND J. T. HAMILTON*

From the Department of Pharmacology, University of Edinburgh

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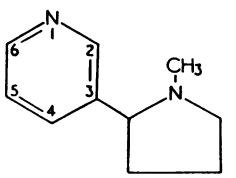
A number of isomers and homologues of nicotine, (2-, 3- and 4-pyridylmethyl)- and [2-(2-, 3- and 4-pyridyl)ethyl]- dialkylamines and trialkylammonium salts, have been prepared. They have been tested for their ability to act like acetylcholine in causing contracture of the chick biventer-cervicis and, some of them, for their ability to stimulate the superior cervical ganglion of the cat, causing contracture of the nictitating membrane. All the compounds have been tested for their ability to block transmission on the superior cervical ganglion of the cat and on the rat diaphragm preparation, and most of them for ability to inhibit the enzymatic hydrolysis of acetylcholine, using an acetone-powder of dog caudate nucleus as a source of acetylcholinesterase. The dissociation constants of the compounds have been measured by electrometric titration. The dissociation constants were used to compute the amount of monovalent ion present in the conditions of the biological tests, and the activities of the compounds have, accordingly, been compared on an ionic, as well as on a molecular, basis. The two sets of figures do not differ greatly. Trimethyl[2-(3-pyridyl)ethyl]ammonium (23) was the most potent compound on the chick and cat preparations. On an ionic basis (that is, compared with the monovalent nicotinium ion) this was 2.6 times as active as nicotine on the chick biventer and 11 times as active on the cat superior cervical ganglion. On the rat diaphragm it was 7.1 times as active as nicotine and less active than 1-methyl-1-(3-pyridylmethyl)-pyrrolidinium (26) (9.5 times the nicotinium ion) and trimethyl(4-pyridylmethyl)-ammonium (21) (11 times). The relationships between structure and dissociation constant, anticholinesterase activity, and activity in the pharmacological tests have been discussed.

Dale (1934) used the term " nicotine-like " to describe the actions of acetylcholine at ganglia and at the neuromuscular junction. Hey (1949, 1952), reviewing the relationships between the chemical structure of esters and ethers of choline and their ability to stimulate ganglia, suggested that activity depended on the presence in a molecule of two features, the onium group and a suitably placed partial positive charge. In Hey's examples this charge was thought to be located on the ether oxygen atom separated by two carbon atoms from the quaternary nitrogen atom. Hey obtained support for this hypothesis from a study of the effects of a number of substituted phenyl ethers of choline on the blood pressure of cats, anaesthetized with chloralose and treated with atropine. Electron-withdrawing substituents on the benzene ring, which would increase the partial positive charge on the ether

* Present address: Department of Pharmacology, University of Western Ontario, London, Ontario, Canada.

oxygen atom, appeared to increase nicotine-like activity, whereas electron-releasing substituents reduced it.

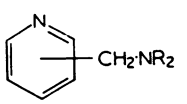
Ormerod (1956) tested a number of substituted benzoic esters of choline for nicotine-like activity on several preparations (rise in blood pressure in the anaesthetized cat after atropine, stimulation of the superior cervical ganglion, contracture of the frog rectus and block of transmission in the rat phrenic nerve-diaphragm preparation). In this series of compounds, however, electron-withdrawing substituents decreased activity whereas electron-releasing substituents increased it. The effect of the substituent on the ether oxygen atom of the ester group, however, must be relayed through the carbonyl portion, which is itself strongly polarized, the carbonyl oxygen atom being partially negative and the carbon atom partially positive. In these circumstances there can be no considerable positive charge on the adjacent ether oxygen atom: Hey, in fact, found that benzoylcholine has only 2½% of the activity of choline phenyl ether (although this estimate does not allow for any destruction of the benzoylcholine by cholinesterase). Further, the substitution of electron-releasing groups in the benzene ring would decrease the partial positive charge on the carbonyl carbon atom and hence should favour activity: electron-withdrawing substituents, by increasing still further the polarization of the carbonyl group, should decrease activity. Ormerod's apparently contradictory results, therefore, can in fact be explained by Hey's hypothesis.



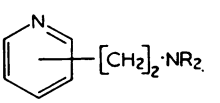
I

In nicotine (I) itself the carbon atoms at positions 2, 4 and 6 of the pyridine ring are deficient in electrons. The relative net charges in the pyridine ring, calculated by the molecular orbital method of Longuet-Higgins & Coulson (1947), are illustrated in Fig. 1. At physiological *p*H, nicotine is mostly in the form of the monovalent ion (Taylor, 1951). The pharmacological activity of nicotine could therefore be ascribed to two features, the onium group and the positive charge on the carbon atoms at positions 2 and/or 4 and/or 6 of the pyridine ring. The two optical isomers of nicotine, *R* and *S*, appear to be approximately equiactive, which supports this view (review by Barlow, 1960).

A number of isomers and homologues of nicotine have therefore been prepared and tested for their effects on transmission at the neuromuscular junction and in ganglia. The compounds were 2-, 3- and 4-pyridylmethyl- and 2-(2-, 3- and 4-pyridyl)ethyl- dialkylamines:

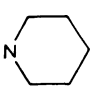


and



where $NR_2 = NMe_2$,

and



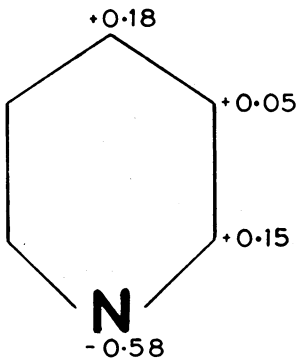


Fig. 1. Relative net charge differences in the pyridine ring (from Longuet-Higgins & Coulson, 1947).

and some quaternary metho-salts. They were tested on the rat-diaphragm (Bülbring, 1946), the chick biventer-cervicis (Ginsborg & Warriner, 1960), the superior cervical ganglion of the cat (Paton & Perry, 1953), and, manometrically, as inhibitors of acetylcholinesterase. Their dissociation constants were measured by electrometric titration.

2-(2-Pyridyl)ethyldialkylamines have been tested as antihistamines by Zamboni & Vitali (1956) and found to be inactive. The compounds synthesized for this study are set out in Table 1. Numbers in parenthesis following compounds named in the text correspond with the compound number in Table 1 or Table 3.

METHODS

Chemical methods

Compounds. Melting-points (taken on a Kofler hot stage) and analytical results are listed in Table 1. Dimethyl[2-(2-pyridyl)ethyl]amine (4) was prepared by Zamboni & Vitali (1956), but only the boiling-point is recorded and no analytical figures are given. Reich & Levine (1955), however, obtained a dipicrate, m.p. 177–8° C (*c.f.* 184.5–185.5° C, Table 1), and also a dipicrate of 1-[2-(2-pyridyl)ethyl]pyrrolidine (10), m.p. 158.5–159.5° C (*c.f.* 162–3° C, Table 1). Magnus & Levine (1956) obtained a dipicrate of dimethyl[2-(4-pyridyl)ethyl]amine (6), m.p. 159.5–160.5° C (*c.f.* 166–7° C, Table 1), and of 1-[2-(4-pyridyl)ethyl]pyrrolidine (12), m.p. 169–170° C (*c.f.* 157–8° C, Table 1).

Synthetic methods

The *pyridylmethyldialkylamines* were prepared by heating the appropriate pyridylmethyl chloride hydrochloride in ethanol for approximately 8 hr under reflux with an excess (4 moles) of the appropriate secondary base. The solvent and unreacted base were distilled off under vacuum on a steam-bath. The residue was dissolved in water, made alkaline with solid sodium hydroxide and extracted with ether. The ether extract was dried with anhydrous potassium carbonate (or sodium sulphate) and distilled. The dihydrobromide was obtained by the addition of excess hydrogen bromide dissolved in ethanol; the product was then concentrated under reduced pressure and treated with suitable solvent or mixture of solvents (methanol, ethanol, ethyl methyl ketone and/or ether) until it crystallized, and was recrystallized until the melting point was constant.

2-(2-Pyridyl)ethyldialkylamines were prepared in an identical manner from 2-(2-pyridyl)ethyl chloride hydrochloride.

TABLE 1

MELTING POINTS AND ANALYSES

Compound no.	Point of attachment of pyridine ring	Dihydrobromide m.p.	Dihydrobromide Cryst.	Dihydrobromide C	Dihydrobromide H	Dipicrate m.p.	Dipicrate Cryst.	Dipicrate C	Dipicrate H
1	α	184–6°	EtOH	32·4	4·71	175–6° dec.	EtOH	40·5	3·00
2	β	221–2°	MeOH/EtOH/EMK	32·3	4·51	203–4°	EtOH	40·5	2·96
3	γ	233–7° dec.	MeOH/EtOH	32·5	5·11	212–14° dec.	EtOH/H_2O	40·7	3·21
		$C_8H_{14}N_2Br_2$	requires	32·2	4·74	$C_{20}H_{18}N_8O_{14}$	requires	40·4	3·06
4	α	193–4°	MeOH/EtOH	34·8	5·49	184·5–185·5	Dioxan	41·5	3·23
5	β	202–3°	EtOH	34·5	4·97	181–2°	EtOH/H_2O	41·7	3·11
6	γ	245–9° dec.	MeOH	34·4	5·38	166–7°	Dioxan/H_2O	41·8	3·22
		$C_9H_{16}N_2Br_2$	requires	34·6	5·13	$C_{21}H_{20}N_8O_{14}$	requires	41·5	3·32
7	α	Sinters 172, melts 187–8°	EtOH	37·1	5·27	190·5–191° dec.	EtOH/H_2O	42·7	3·49
8	β	206–9°	EtOH	37·1	4·79	192–3° dec.	EtOH/H_2O	42·6	3·27
9	γ	187–8° dec.	EtOH	37·2	5·24	Dec. above 200°, melts 212–14°	EtOH/H_2O	42·5	3·39
		$C_{10}H_{16}N_2Br_2$	requires	37·0	4·99	$C_{22}H_{26}N_8O_{14}$	requires	42·6	3·22
10	α	176–8°	EtOH	39·2	5·72	162–3°	Dioxan	43·6	3·25
11	β	180–2°	EtOH/acetone	38·9	5·67	197–8° dec.	EtOH/H_2O	43·5	3·76
12	γ	244–6° dec.	EtOH	38·8	5·52	157–8°	Dioxan/H_2O	43·3	3·45
		$C_{11}H_{18}N_2Br_2$	requires	39·1	5·37	$C_{23}H_{22}N_8O_{14}$	requires	43·5	3·50
13	α	214–16° dec.	EtOH	39·0	5·29	185–7°	Dioxan	43·8	3·33
14	β	Dec. above 190°, 221–4°	EtOH	39·3	5·46	179–80° dec.	EtOH/H_2O	43·5	3·54
15	γ	Sinters 149–151°, melts 185°	EtOH	39·2	5·70	198–9°	Dioxan	43·8	3·38
		$C_{11}H_{18}N_2Br_2$	requires	39·1	5·37	$C_{23}H_{22}N_8O_{14}$	requires	43·5	3·50
16	α	210–211°	MeOH/EtOH	40·9	6·00	163–4°	EtOH/H_2O	44·6	3·80
17	β	251–4° dec.	EtOH	41·1	5·73	176–8° dec.	EtOH/H_2O	44·4	3·73
18	γ	246–8° dec.	MeOH/EtOH	41·2	6·08	151–2°	EtOH/H_2O	44·9	3·63
		$C_{12}H_{20}N_2Br_2$	requires	40·9	5·74	$C_{24}H_{24}N_8O_{14}$	requires	44·5	3·74

[*Continued overleaf*

TABLE 1—*continued*

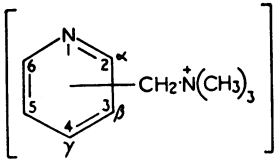
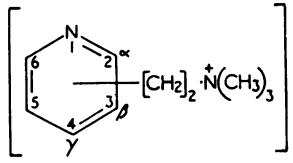
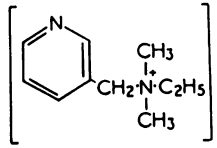
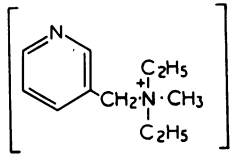
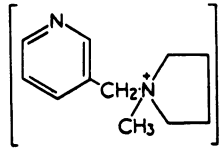
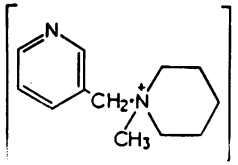
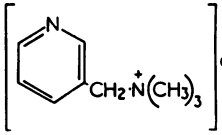
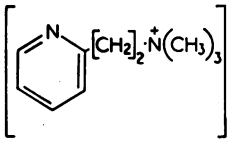
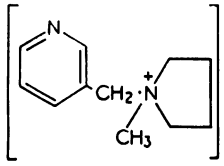
	Compound no.	Point of attachment of pyridine ring	m.p.	Cryst.	C	H	Br⁻
	19	α	180–1°	EtOH	34·4	5·01	50·9, 51·2
	20	β	196·5–197·5	EtOH/Et_2O	34·7	5·03	50·9, 51·0
	21	γ	Dec. above 200°, melts 236–8°	MeOH/EtOH	34·8	4·98	50·9, 51·3
			$C_9H_{16}N_2Br_2$	requires	34·6	5·18	51·3
	22	α	180–1°	EtOH/acetone/Et_2O	36·7	5·35	— —
	23	β	226–7°	EtOH	36·8	5·73	48·5, 48·5
			$C_{10}H_{18}N_2Br_2$	requires	36·8	5·58	49·1
	24	β	192–3°	MeOH/EtOH	37·0	5·24	—
			$C_{10}H_{18}N_2Br_2$	requires	36·8	5·57	
	25	β	190–1°	MeOH/EtOH	39·2	5·81	
			$C_{11}H_{20}N_2Br_2$	requires	38·8	5·94	
	26	β	198–9°	EtOH	39·3	5·27	—
			$C_{11}H_{18}N_2Br_2$	requires	39·1	5·37	—

TABLE 1—*continued*

		Compound no.	Point of attachment of pyridine ring	m.p.	Cryst.	C	H	Br⁻
	Br^-,HBr	27	β	198–9° $C_{12}H_{20}N_2Br_2$	EtOH/Et_2O requires	40·7 40·9	5·16 5·74	44·9 45·5
	dipicrate	(20a)	β	210–11° $C_{21}H_{20}N_8O_{14}$	EtOH/EMK requires	41·5 41·5	3·15 3·32	— —
	dipicrate	(22a)	α	150–1° $C_{22}H_{22}N_8O_{14}$	MeOH/EtOH/H_2O requires	42·8 42·5	3·50 3·57	— —
	dipicrate	(26a)	β	192–3° $C_{23}H_{22}N_8O_{14}$	EtOH/H_2O requires	43·6 43·6	3·56 3·51	— —

Analytical results for nicotine monomethiodide are given in the following paper (Barlow & Hamilton, 1962).

2-(3-Pyridyl)ethyldialkylamines were prepared from 3-pyridylmethyl chloride hydrochloride by way of the nitrile, acid, ester and alcohol.

3-Pyridylacetonitrile

The first stage was a modification of the method of Mosher & Tessieri (1951). 3-Pyridylmethyl chloride hydrochloride (49.2 g, 0.3 mole) dissolved in ethanol (420 ml.) was refluxed for 5 hr with potassium cyanide (45.0 g, 0.69 mole) dissolved in water (90 ml.) and ethanol (450 ml.). Most of the solvent was distilled off under reduced pressure on a steam-bath and the residue was dissolved in water (400 ml.) to which was added solid potassium carbonate (100 g) and the whole shaken with several portions of ether. The combined extracts (2 l.) were dried with anhydrous potassium carbonate and distilled; b.p. 144–148° C/20 mm, $N\ ^{22}_{D}$ 1.5288, yield 56% (20 g). Mosher & Tessieri recorded b.p. 91° C/2 mm, $N\ ^{20}_{D}$ 1.5278, yield 34%. Protiva, Jilek & Pliml (1951) recorded b.p. 144–146° C/20 mm.

3-Pyridylacetic acid and ethyl ester

3-Pyridylacetonitrile (20 g, 0.17 mole) was refluxed with concentrated hydrochloric acid (125 ml.) for 8 hr. The product was concentrated under reduced pressure on a steam-bath and the residue extracted with boiling ethanol (2 portions of 150 ml.). The extract was filtered to remove ammonium chloride and the 3-pyridylacetic acid hydrochloride esterified by the method of Micovic (1937), that is, toluene (100 ml.) and sulphuric acid (3 ml.) were added and the azeotropic mixture of ethanol, toluene and water was distilled off. When the removal of water appeared to be complete, most of the excess ethanol and toluene were distilled off under reduced pressure on a steam-bath and the residue, dissolved in water, was made alkaline to *p*H 9 and extracted with ether. The ether extract was dried with anhydrous sodium sulphate and distilled, b.p. 130–132° C/10 mm., $N\ ^{21}_{D}$ 1.5002, yield 88% (24.5 g). Protiva, Jilek & Pliml (1951) recorded b.p. 110–111° C/2.5 mm.

2-(3-Pyridyl)ethanol

Ethyl 3-pyridylacetate (24.5 g, 0.15 mole) dissolved in ether (25 ml.) was added slowly to lithium aluminium hydride (5 g, 0.13 mole) in ether (300 ml.). When the addition was complete, the mixture was heated under reflux for 30 min, allowed to cool and treated carefully with water (50 ml.). The ether layer was decanted off and the residue extracted with boiling benzene (3 portions of 200 ml.). The combined extracts were dried with anhydrous potassium carbonate and distilled, b.p. 151–152° C/10 mm, $N\ ^{21}_{D}$ 1.5390, yield 74% (13.5 g). Dornow & Schacht (1947) recorded b.p. 144°/10 mm.

2-(3-Pyridyl)ethyl chloride

2-(3-Pyridyl)ethanol, dissolved in chloroform, was added with stirring to thionyl chloride dissolved in ether, cooled in ice-water. The product (formed in almost quantitative yield) was recrystallized from ethanol and ether and used for the preparation of the tertiary bases in exactly the same manner as that described for the pyridylmethyldialkylamines and 2-(2-pyridyl)-ethyldiakylamines. A small portion of the 2-(3-pyridyl)ethyl chloride hydrochloride, recrystallized twice more, had m.p. 157–8° C; found C, 46.8; H, 4.54; $C_7H_9NCl_2$ requires C, 47.2; H, 5.10%.

2-(4-Pyridyl)ethyldialkylamines were prepared by the addition of the appropriate secondary amine to 4-vinylpyridine as described by Magnus & Levine (1956). The dihydrobromide was prepared from the base as described above.

The quaternary salts were prepared by heating the appropriate pyridylalkyl chloride hydrochloride with an excess (4 moles) of tertiary base (trimethylamine, ethyldimethylamine, diethylmethylamine, 1-methylpyrrolidine or 1-methylpiperidine) for 5 hr under reflux. The solvent and excess amine were distilled off under reduced pressure on a steam-bath. The residue was dissolved in a small volume of water and passed down a column of anion-exchange resin

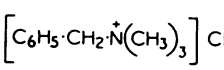
(IRA 400). The aqueous solution containing the quaternary hydroxide was then warmed to 40° C under reduced pressure and some water and excess tertiary base distilled off. When the volume had been reduced by about one-third, it appeared that only traces of tertiary base remained and the solution was made strongly acid with concentrated aqueous hydrogen bromide and concentrated under reduced pressure on a steam-bath. The quaternary salt usually crystallized when the gummy residue was covered with ethyl methyl ketone and/or ethanol. It was recrystallized as necessary. Although 1-methyl-1-(3-pyridylmethyl)-pyrrolidinium bromide (26) was obtained in good yield by this method from 1-methylpyrrolidine, the analogous piperidine derivative (27) was formed only in poor yield using 1-methylpiperidine.

Other compounds

Benzyltrimethylammonium chloride (II) (28) had m.p. 238.5–239.5° C (dec): found, Cl^-, 19.2; calculated for $C_{10}H_{16}NCl$, 19.1%.

Trimethylphenethylammonium bromide (III) (29) had m.p. 242.5–243.5° C; found, Br^-, 32.3; calculated for $C_{11}H_{18}NBr$, 32.5%.

Choline phenyl ether (IV) (30) and homocholine phenyl ether (V) (31), prepared by the method of Hey (1952), had m.p. 169° C and 153° C respectively. Renshaw & Armstrong (1933) recorded m.p. 167° C and 156° C.



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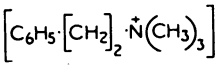

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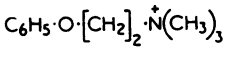

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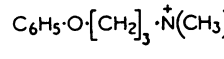


V

Preparations. The rat diaphragm was dissected out as described by Bülbring (1946) and suspended in Krebs bicarbonate solution in a 25 ml. bath at 37° C, gassed with oxygen (95%) and carbon dioxide (5%). The phrenic nerve was stimulated maximally with square-wave shocks of 0.75 msec duration at a rate, usually, of 5 per min. The contractions of the muscle were recorded using a semi-isometric torsion-lever (Condon, 1957), writing on a smoked drum.

The chick biventer-cervicis was dissected out and mounted in exactly the same way as described by Ginsborg & Warriner (1960). The volume of the bath was 15 ml., but the conditions were otherwise exactly the same as those, described above, for the rat diaphragm.

The cat superior cervical ganglion preparation was set up as described by Paton & Perry (1953). The preganglionic sympathetic nerve was stimulated maximally with square-wave shocks of 0.75 msec duration at a rate of 10/sec, and the contraction of the right nictitating membrane was recorded on a smoked drum. Injections were made retrogradely into the lingual artery or, if this were too small, retrogradely into the external carotid artery. In most experiments the blood pressure was also recorded.

The caudate nucleus of the dog was used as a source of acetylcholinesterase in the manometric experiments. An acetone-powder was prepared from the caudate nuclei of 5 dogs and stored at room temperature over calcium chloride.

Experimental procedures: (1) *Measurement of activity at the neuromuscular junction.* Nicotine-like stimulant activity can be estimated on the chick biventer-cervicis by observing the contracture produced. This is a response of the slow fibres in the muscle and is associated with depolarization of the end-plate (Ginsborg, 1960). The drugs were added to the bath from a blow-out pipette and allowed to act until a maximum effect was produced (about 10 min). The preparation was then washed three times and left to recover for the same length of time that the drug had been left in the bath.

Nicotine was used as a standard in all the experiments, and the activity of the test substance was estimated by comparing the concentrations producing comparable contractures. At least

two dose levels of nicotine and of the test substance were used and the graphs of the logarithm of the dose against the height of contracture were plotted. These appeared to be approximately parallel, and from them the equipotent molar ratio was obtained. In some experiments the equipotent molar ratio was obtained by a full four-point assay.

Nicotine-like stimulant activity cannot conveniently be measured on the rat diaphragm preparation, which was accordingly used only to assess blocking activity. The drugs were added to the bath from a blow-out pipette and allowed to act for 10 min, at the end of which period the block was only increasing slightly with the doses used. The preparation was then washed three times and left to recover for 10 min. Nicotine was used as a standard in all the experiments, and the activity of the test substance was estimated by comparing the concentrations producing comparable degrees of block. At least two dose levels of nicotine and of the test substance were used, and the graphs of the logarithm of the dose against the percentage reduction of the contractions were compared. These were usually approximately parallel and could be used to derive the equipotent molar ratio. In some instances, however, they were not parallel, and the value of the equipotent molar ratio quoted refers to concentrations producing 50% block and cannot have much meaning. In some experiments the equipotent molar ratio was obtained by a full four-point assay.

(2) *Measurement of activity at the ganglion.* Stimulant activity was estimated in some experiments (in the absence of electrical stimulation of the preganglionic nerve) by observing the contracture of the nictitating membrane produced by a dose of a drug. The volume injected (0.1 ml.) was kept constant and was followed by an injection of 0.2 ml. saline with a further 0.2 ml. if necessary. The dose was altered by diluting the stock solution of the drug suitably with saline. Nicotine was used as a standard and the equipotent molar ratio for the test drug calculated as described above for the experiments with the chick biventer.

Blocking activity was estimated for all the compounds by observing the decrease in the contracture of the nictitating membrane (in response to electrical stimulation of the preganglionic nerve) produced by a dose of the drug. The injection was given as described above and the equipotent molar ratio calculated as described for the experiments with the rat diaphragm.

(3) *Inhibition of the hydrolysis of acetylcholine by the acetylcholinesterase of dog caudate nucleus.* This was studied manometrically (Ammon, 1934). The acetone-powder of dog caudate nucleus was suspended in Krebs bicarbonate buffer solution and homogenized by hand. In a preliminary series of experiments the effect of substrate concentration upon the initial rate of evolution of carbon dioxide was determined using 3 mg, 4 mg and 6 mg of tissue per flask. From the results (summarized in Table 2) the Michaelis-Menten constant for the enzyme was calculated and found to be 3.4×10^{-4}. Augustinsson (1948) obtained the value 2.9×10^{-4} from the dog brain at 37° C.

The effects of inhibitors were then assessed by measuring the percentage inhibition of the hydrolysis of 10^{-3} M acetylcholine. Flasks were made up as follows:

I. Thermobarometer containing water only.
II. Blank, containing buffer, acetone-powder but no substrate.
III. Control, containing buffer, acetone-powder and substrate.
IV. Blank, containing buffer, acetone-powder and inhibitor, but no substrate.
V. Test, containing buffer, acetone-powder, inhibitor and substrate.

The temperature was 37° C, the gas phase nitrogen (95%) and carbon dioxide (5%), each flask contained 4 mg of acetone-powder and the total volume of fluid was 6 ml. From a graph of the percentage inhibition against the logarithm of the concentration of the inhibitor, the *p*I 50 was calculated. This, however, varies with substrate concentration. Assuming a competitive inhibition, $1+S/K_s = K/K_i$, where S is the substrate concentration and K_s the Michaelis-Menten constant, I the inhibitor concentration producing 50% block and K_i the inhibitor constant. In these experiments $S/K_s = 2.9$ and hence

$$-\log K_i = p\text{I } 50 + 0.59.$$

TABLE 2

EFFECT OF CONCENTRATION ON THE RATE OF HYDROLYSIS OF ACETYLCHOLINE BY ACETONE-POWDERED DOG CAUDATE NUCLEUS

Enzyme tissue/ flask	μl. carbon dioxide evolved/30 min at different substrate concentrations (molar)					
	0·01	0·005	0·0025	0·001	0·0005	0·00025
3 mg	50	55	55	50	40	30
4 mg	60	66	66	60	48	33
6 mg	93	102	102	99	78	54

The rate of hydrolysis was determined by plotting the evolution of carbon dioxide against time and measuring the slope of the (initial) linear part of the curve. For comparison the slope is expressed as μl. evolved in 30 min. To obtain the Michaelis-Menten constant, the reciprocal of the rate (1/V) was plotted against the reciprocal of the substrate concentration (1/S). The values, shown below, for the two lowest concentrations of substrate all give a Michaelis-Menten constant of $3{\cdot}4 \times 10^{-4}$

Tissue/ flask	Substrate conc. (S) (molar)	1/S	1/V
3 mg	0·001	1,000	0·60
	0·0005	2,000	0·75
	0·00025	4,000	1·00
4 mg	0·001	1,000	0·50
	0·0005	2,000	0·63
	0·00025	4,000	0·91
6 mg	0·001	1,000	0·30
	0·0005	2,000	0·38
	0·00025	4,000	0·56

(4) *Dissociation constants.* These were determined by electrometric titration using the method of Albert & Goldacre (1943). The dihydrobromide (10 ml. of an M/100 solution + 10 ml. deionized water) was titrated with standard sodium hydroxide (N/10) using a glass electrode and a calomel half-cell connected to a Marconi pH meter (Type TF 511 D). The solution was maintained at 25° ± 0.1° C and stirred vigorously with a compressed-air driven stirrer (Quickfit MU 8/0). The pH after the addition of 0.5 ml. alkali gives the pK_a for the dissociation of the proton attached to the nitrogen atom in the pyridine ring, and after 1.5 ml. alkali it gives the pK_a for the dissociation of the proton attached to the side-chain amino-group. For the compounds with a quaternary side-chain, titration of the bromide hydrobromide with alkali gives the pK_a for the dissociation of the proton attached to the nitrogen atom in the pyridine ring (after the addition of 0.5 ml. N/10 sodium hydroxide). The pK_a at 37° C was calculated from these results by the method of Albert (1952).

RESULTS

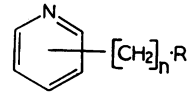
The equipotent molar ratios for stimulant activity on the chick biventer-cervicis, for blocking activity on the rat diaphragm, and for stimulant and blocking activity on the superior cervical ganglion of the cat are set out in Table 3. This also gives estimates of the logarithm of the reciprocal of the inhibitor constant for the hydrolysis of acetylcholine by the cholinesterase of dog caudate nucleus, and indicates the number and type of experiment on which all the results are based.

It is only possible to express the activity of a compound relative to that of nicotine as a number if the time-course of the effect produced by it is similar to that produced by nicotine and if the dose-response curve of the drug is parallel to that of nicotine. In most instances this appeared to be true (by inspection of the kymograph records and of the log dose-response curves), but there were some exceptions. As blocking agents on the cat superior cervical ganglion the effects of

TABLE 3

EFFECTS OF ANALOGUES OF NICOTINE (EQUIPOTENT RATIOS)

Figures in *italics* indicate the number of experiments, *a* being an estimation from the log dose/effect graphs and *b* a 4-point assay



Point of attachment	n=	R=	Compound no.	Chick. Biventer cervicis contracture			Rat. Diaphragm block			Cat. Superior cervical ganglion: Stimulation			Cat. Superior cervical ganglion: Block			Effects on cholinesterase of dog caudate nucleus pK_i		
				Molar	Ionic		Molar	Ionic		Molar	Ionic		Molar	Ionic		Molar	Ionic	
β	2	Trimethyl-ammonium	23	0·28	0·39	*1a*	0·10	0·14	*1a*	—	—	—	0·063	0·088	*3b*	1·11	1·11	*1*
γ	1	Trimethyl-ammonium	21	1·00	1·4	*1a*	0·063	0·088	*1a*	—	—	—	17	24	*2a*	—	—	—
β	1	Trimethyl-ammonium	20	1·00	1·4	*1a*	0·18	0·25	*1a*	0·50	0·70	*1a*	0·70	0·98	*1a 2b*	—	—	—
Monomethylnicotinium				1·2	1·7	*2a*	0·11	0·15	*1b*	3·5	4·9	*1b*	4·3	6·0	*3a 1b*	3·19	3·19	*2*
β	1	1-Methyl-pyrrolidinium	26	1·5	2·1	*2a*	0·075	0·10	*1a*	—	—	—	2·8	3·9	*3a*	3·16	3·16	*2*
β	1	Pyrrolidino	8	1·7	2·0	*3a 1b*	0·72	0·86	*2b*	—	—	—	0·70	0·84	*2a 2b*	3·29	3·36	*3*
β	1	Piperidino	14	1·8	2·1	*4a*	0·50	0·59	*2a*	—	—	—	2·4	2·8	*1a 1b*	3·04	3·12	*3*
Benzyltrimethyl-ammonium			28	4·2	5·9	*1a*	0·38	0·53	*2a*	—	—	—	29	41	*1a*	—	—	—
β	1	Ethyldimethyl-ammonium	24	4·6	6·4	*1a*	0·37	0·52	*1a*	1·3	1·8	*1a*	9·0	13	*1a 1b*	—	—	—
Trimethylphenethyl-ammonium			29	8·7	12	*1a*	0·30	0·42	*1a*	—	—	—	24	34	*1a 2b*	—	—	—
α	2	Piperidino	16	22	30	*1a 2b*	0·43	0·59	*2a*	—	—	—	59	81	*2a*	3·44	3·45	*3*
γ	1	Pyrrolidino	9	22	24	*2b*	2·0	2·2	*1a 1b*	—	—	—	33	37	*3a 1b*	2·64	2·74	*3*

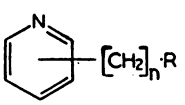
TABLE 3—*continued*

β	1	Diethylmethyl-ammonium	25	25	35	*1a*	1·5	2·1	*1a*	Inactive		*1a*	30	42	*1a* *1b*	—	—	—
β	1	1-Methyl piperidinium	27	25	35	*1a*	0·66	0·92	*1a*	—	—	—	100	140	*1a*	—	—	—
β	1	Dimethylamino	2	28	28	*2a*	8·4	8·5	*3b*	25	25	*1b*	17	17	*1a* *1b*	2·64	2·78	*3*
α	1	Piperidino	13	32	40	*3a*	2·3	2·9	*2a*	—	—	—	35	44	*2a*	3·21	3·26	*3*
β	2	Dimethylamino	5	36	48	*1a* *1b*	8·3	11	*2a*	—	—	—	3·3	4·4	*2a* *1b*	2·93	2·95	*2*
β	2	Piperidino	17	40	53	*2a*	0·65	0·86	*2a*	44	58	*1a*	49	65	*1a*	3·22	3·25	*3*
β	2	Pyrrolidino	11	47	64	*1a* *1b*	1·00	1·4	*2a*	33	45	*1a*	52	71	*1a*	1·61	1·62	*2*
α	2	Trimethyl-ammonium	22	55	77	*1a*	5·0	7·0	*1a*	—	—	—	41	57	*2a*	2·81	2·81	*2*
α	1	Trimethyl-ammonium	19	55	77	*1a*	5·0	7·0	*1a*	—	—	—	63	88	*1a*	1·41	1·41	*2*
γ	1	Piperidino	15	57	53	*2a*	4·1	3·8	*2a*	—	—	—	170	160	*2a*	3·26	3·43	*3*
α	2	Pyrrolidino	10	62	85	*1a* *1b*	0·89	1·3	*1a* *1b*	80	110	*1a*	54	74	*2a*	4·18	4·19	*2*
α	1	Pyrrolidino	7	91	110	*2a* *1b*	6·7	8·5	*1a* *4b*	—	—	—	110	140	*2a*	3·57	3·62	*3*
α	2	Dimethylamino	4	100	130	*2a*	16	21	*3a*	2·4	3·2	*1a*	67	88	*2a*	4·32	4·35	*2*
γ	1	Dimethylamino	3	320	240	*2a*	11	8·4	*4b*	400	310	*1a*	400	310	*1a*	1·58	1·84	*2*
α	1	Dimethylamino	1	670	730	*2a*	37	40	*1a* *1b*	—	—	—	110	120	*2a*	3·23	3·34	*2*
γ	2	Dimethylamino	6	1,100	1,400	*3a*	22	29	*3a*	—	—	—	240	310	*2a*	2·58	2·61	*2*
γ	2	Pyrrolidino	12	>4,000	>4,000	*2a*	2·1	2·9	*2a*	>800	>1,100	*1a*	330	450	*2a*	2·85	2·86	*2*
γ	2	Piperidino	18	>6,000	>6,000	*2a*	1·4	1·9	*2a*	—	—	—	360	490	*3a*	2·97	2·98	*3*
Choline phenylether			30	—	—	—	—	—	—	0·18	0·25	*1b*	0·47	0·66	*1b*	—	—	—
Homocholinephenylether			31	—	—	—	—	—	—	Inactive		*1a*	3·0	4·2	*1b*	—	—	—
Nicotine				1	1	—	1	1	—	1	1	—	1	1	—	3·18	3·33	*3*

certain 2- and 4-substituted compounds, dimethyl(2-pyridylmethyl)amine (1), dimethyl[2-(2-pyridyl)ethyl]amine (4), 1-(2-pyridylmethyl)piperidine (13), 1-[2-(2-pyridyl)ethyl]piperidine (16), dimethyl[2-(4-pyridyl)ethyl]amine and 1-[2-(4-pyridyl)-ethyl]pyrrolidine (12), were much slower in onset and more prolonged than those of nicotine. The log dose-response curves for certain quaternary derivatives, trimethylphenethylammonium (29), trimethyl(4-pyridylmethyl)ammonium (21), diethylmethyl-(3-pyridylmethyl)ammonium (25), choline phenyl ether (30) and homocholine phenyl ether (31), were also flatter than those of nicotine. On the chick biventer cervicis the log dose-response curves for 1-(2-pyridylmethyl)pyrrolidine (7) and 1-[2-(2-pyridyl)ethyl]pyrrolidine (10) were much flatter than that of nicotine, but on the rat diaphragm the log dose-response curves for these compounds, and also for the corresponding dimethylamine derivatives, dimethyl(2-pyridylmethyl)amine (1), and dimethyl[2-(2-pyridyl)ethyl]amine (4), were steeper than the log dose-response curve

TABLE 4

pK_a VALUES AND % IONIZATION OF COMPOUNDS

Point of attachment	n=	 R=	Compound no.	pK_a pyridine nitrogen atom 25° C.	pK_a side-chain nitrogen atom 25° C.	pK_a side-chain nitrogen atom 37° C.	% ionized form at 37° C. and pH 7·38
α	1	Dimethylamino	1	2·64	8·16	7·92	77·7
β	1	Dimethylamino	2	3·23	8·04	7·80	72·4
γ	1	Dimethylamino	3	3·45	7·70	7·46	54·6
α	2	Dimethylamino	4	3·52	8·79	8·55	93·6
β	2	Dimethylamino	5	4·36	8·90	8·66	95·0
γ	2	Dimethylamino	6	4·72	8·74	8·50	93·0
α	1	Pyrrolidin-1-yl	7	2·60	8·60	8·36	90·5
β	1	Pyrrolidin-1-yl	8	3·20	8·40	8·16	85·7
γ	1	Pyrrolidin-1-yl	9	3·44	8·20	7·96	79·2
α	2	Pyrrolidin-1-yl	10	3·66	9·43	9·19	98·4
β	2	Pyrrolidin-1-yl	11	4·34	9·32	9·08	98·0
γ	2	Pyrrolidin-1-yl	12	4·71	9·31	9·07	98·0
α	1	Piperidino	13	2·67	8·55	8·31	89·4
β	1	Piperidino	14	3·22	8·34	8·10	84·0
γ	1	Piperidino	15	3·96	7·92	7·68	66·7
α	2	Piperidino	16	3·65	9·33	9·09	98·1
β	2	Piperidino	17	4·31	8·85	8·61	94·4
γ	2	Piperidino	18	4·74	9·10	8·86	96·8
α	1	Trimethylammonium	19	2·69			
β	1	Trimethylammonium	20	3·10			
γ	1	Trimethylammonium	21	3·24			
α	2	Trimethylammonium	22	3·54			
β	2	Trimethylammonium	23	4·35			
β	1	Ethyldimethylammonium	24	3·11			
β	1	Diethylmethylammonium	25	3·12			
β	1	1-Methylpyrrolidinium	26	3·13			
β	1	1-Methylpiperidinium	27	3·13			
Monomethylnicotinium				3·15			
Nicotine				3·10	8·01	7·77	71·5

for nicotine. For the majority of compounds, however, especially the more active ones, the graphs appeared near enough parallel to those of nicotine to justify the expression of the activity numerically as in Table 3.

The dissociation constants of the compounds are listed in Table 4 ; each value is the mean of two estimations. From these it is possible to calculate the proportion of the substance present as the univalent ion at 37° C and pH 7.38 and hence to obtain the equipotent ionic ratios, based on the activity per univalent ion rather than on the activity per molecule of salt. The two sets of values are compared in Table 3.

On the chick biventer cervicis only one compound was more active than nicotine, trimethyl[2-(3-pyridyl)ethyl]ammonium bromide (23), which, on an ionic basis, was 2.6 times as active. The trimethyl(3- and 4-pyridylmethyl)ammonium compounds (20, 21) were equiactive and 0.71 times as active as nicotine (per ion) but 4.2 times as active as trimethylphenylammonium.

On the rat diaphragm many compounds were more active than nicotine. The most active were trimethyl(4-pyridylmethyl)ammonium (21) (11 times as active as the nicotinium ion), 1-methyl-1-(3-pyridylmethyl)pyrrolidinium (26) (9.5 times) and trimethyl[2-(3-pyridyl)ethyl]ammonium (23) (7.1 times).

On the cat superior cervical ganglion the most active blocking agents were trimethyl[2-(3-pyridyl)ethyl]ammonium (23) (11 times as active as the nicotinium ion), 1-(3-pyridylmethyl)pyrrolidine (8) (1.2 times) and trimethyl(3-pyridylmethyl) ammonium (20) (1.0 times): the γ-isomer, trimethyl(4-pyridylmethyl)ammonium (21) was only feebly active (0.04 times). Estimates of activity based on ability to stimulate the ganglion seem, from the results available, to agree quite well with the estimates of activity based on ability to block transmission.

On the acetylcholinesterase of the dog caudate nucleus the α-substituted compounds were the most active inhibitors (Table 3), but even the most active of these, dimethyl[2-(2-pyridyl)ethyl]amine (4), has less than 0.1% of the activity of eserine. It is remarkable that quaternary metho-salts of the dimethylamine derivatives are less active than the tertiary bases.

Effects of the position of attachment of the pyridine ring are shown in Fig. 2, in which the position in the ring is plotted against the logarithm of the equipotent molar ratio. On the chick and cat preparations the β-isomer in the pyridylmethyl series of tertiary compounds is invariably the most active. On the rat diaphragm the differences are not so marked. In the pyridylethyl series, considerable activity is shown by the α-compounds. On the chick the γ-compounds are all only feebly active, as is also dimethyl[2-(4-pyridyl)ethyl]amine (6) on the cat. On the rat diaphragm, however, differences between the isomers are not marked, nor are they in the pyrrolidine and piperidine derivatives on the cat (which are only feebly active in absolute terms).

In the quaternary salts the β-compounds, in both the pyridylmethyl and pyridylethyl series, are more active than the α-compounds on all the preparations, but surprisingly high relative activity is shown by trimethyl(4-pyridylmethyl)ammonium (21) on the chick and the rat but not on the cat.

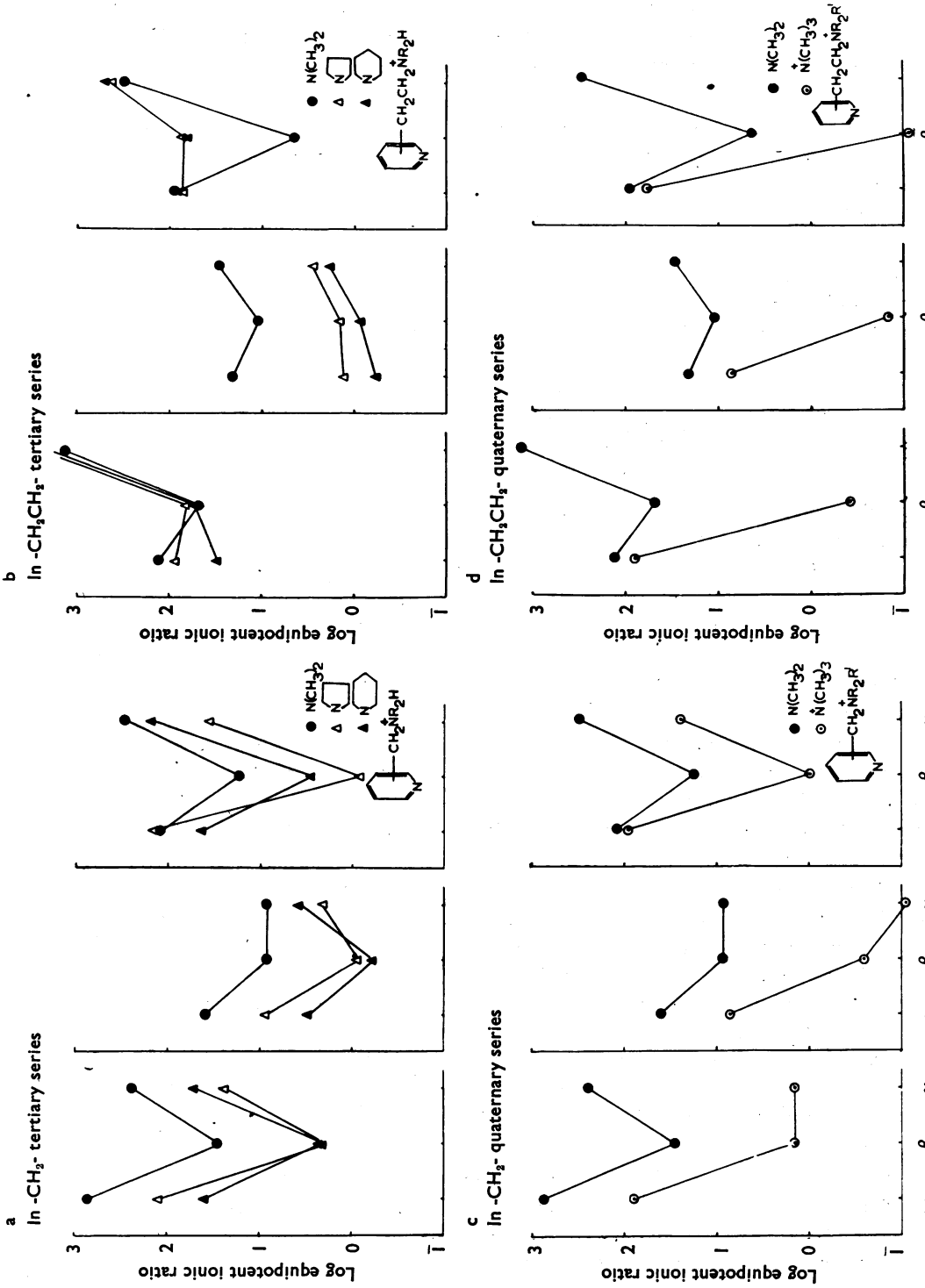


Fig. 2. Effect on activity of the point of attachment to the ring. Ordinate, logarithm of the number of ions of the compound equivalent to one of the nicotinium ion; abscissa, position of attachment to the pyridine ring. A negative value indicates activity greater than the nicotinium ion; high positive values indicate low activity.

Effects of lengthening the chain are shown in Fig. 3, in which the position in the ring is plotted against the logarithm of the number of ions of the pyridylethyl derivative equivalent to one of the corresponding pyridylmethyl derivative. In all the series the extra methylene group increases the activity of the α-compounds by anything up to a factor of 7, with the exception of the piperidine derivatives (in which activity on the cat is slightly reduced) and the quaternary compounds, in which activity on the chick and rat is unaltered. With the β-compounds activity on the rat is little affected, but in the pyrrolidine and piperidine series on the chick and cat it is markedly reduced, whereas in the dimethylamine series it is only slightly reduced on the chick and increased 4-fold on the cat. In the β-trimethylammonium compounds the extra methylene group increases activity, particularly on the cat. With the γ-compounds chain lengthening decreases activity on the chick and the cat, but on the rat, although activity is decreased in the dimethylamino-series, it is actually increased in the piperidino-series.

Effects of quaternization of the side-chain amino-group are shown in Fig. 4, in which the position in the ring is plotted against the logarithm of the number of ions of the quaternary metho-compound equivalent to one of the tertiary compound (as cation). It will be seen that methylation of the dimethylamino-compounds invariably leads to an increase in activity, in some instances more than 100-fold. In the pyrrolidine and piperidine series, as with nicotine itself, activity may increase on the rat but decreases on the chick and to an even greater extent on the cat.

Effects of altering the side-chain amino-group are shown in Fig. 5, in which the identity of the amino-group is plotted against the logarithm of the number of ions of the compound equivalent to one of the analogous dimethylamino-derivative (as cation). The change from dimethylamino to piperidino, pyrrolidin-1-yl, and trimethylammonium generally increases activity in the 3- and 4-pyridylmethyl compounds. In the pyridylethyl series the pyrrolidine and piperidine derivatives may be less active than the dimethylamino-analogue, but the trimethylammonium compound is invariably more active. In general the changes in structure affect activity on the chick and the cat in a similar way, but the activity on the rat may be altered differently. The effects of altering the side-chain amino-group in the α-compounds are rather different, but the situation is here complicated by the low activity of the compounds and by some of them possessing significant anticholinesterase activity.

Effects of replacing the pyridyl group by the phenyl group are shown in Fig. 6, in which the position in the ring is plotted against the logarithm of the number of ions of the phenyl compound equivalent to one of the pyridyl analogue. Replacement of the 2-pyridyl group by the phenyl group in both the arylmethyl and arylethyl series increases activity on the chick and rat considerably, but on the cat only slightly. Replacement of the 3- or 4-pyridyl group by the phenyl group reduced activity, in the cat more than in the rat and in the arylethyl series more than in the arylmethyl series.

Effects of replacing the methyl groups in trimethyl(3-pyridylmethyl)ammonium by ethyl groups are shown in Fig. 7, in which the number of ethyl groups is plotted against the logarithm of the number of ions of the substituted compounds equivalent to one of the trimethylammonium compound. The general decline in activity with

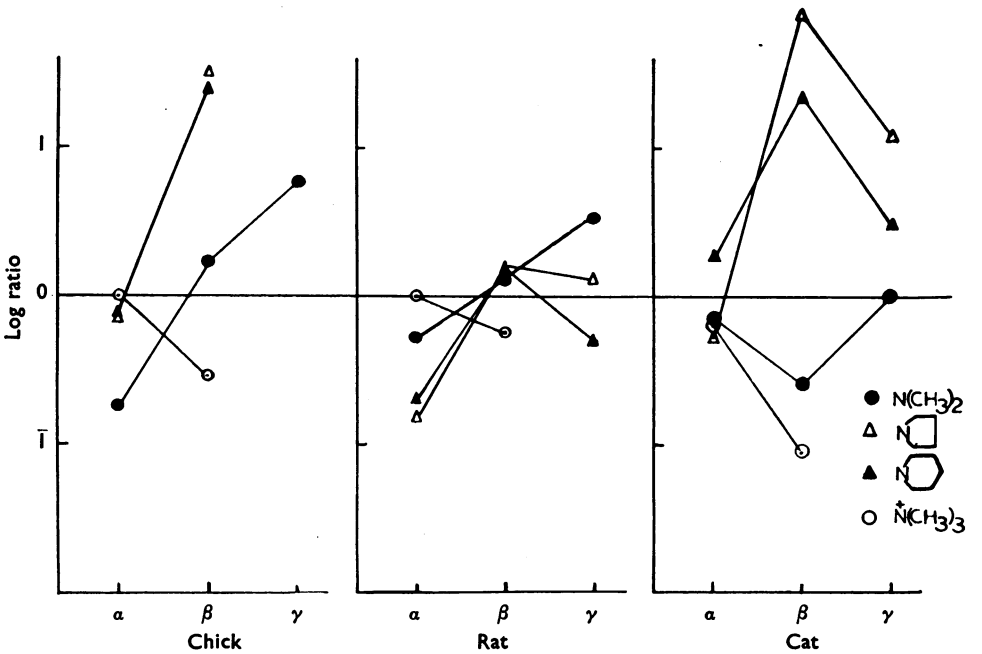


Fig. 3. Effect on activity of chain-length. Ordinate, logarithm of the number of ions of the ethylene derivative equivalent to one of the methylene analogue; abscissa, point of attachment to the pyridine ring. A negative value indicates that activity is increased by lengthening the chain.

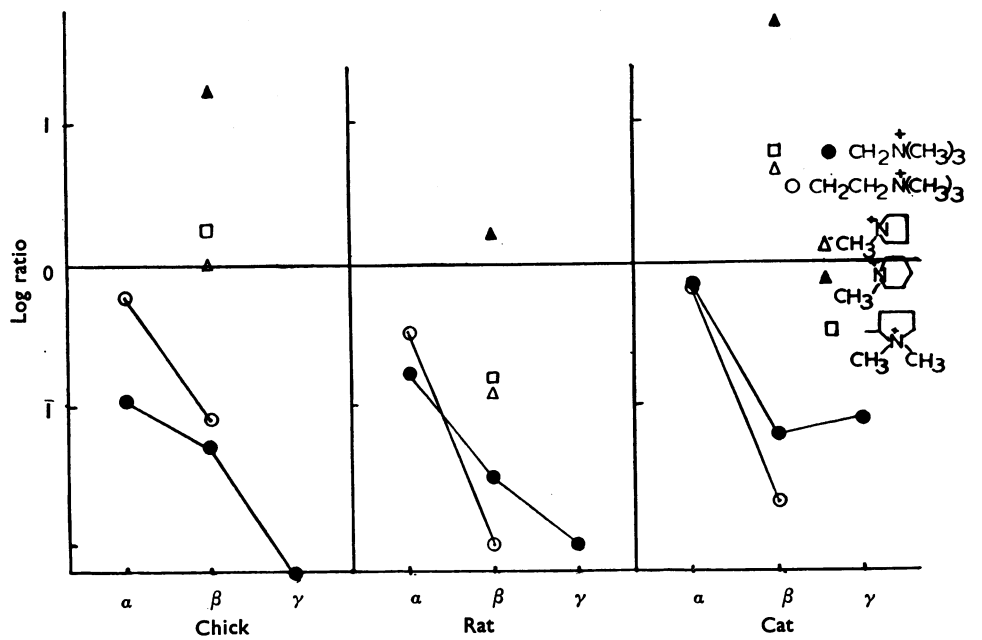


Fig. 4. Effect on activity of quaternization of the side-chain amino-group. Ordinate, logarithm of the number of ions of the metho-salt equivalent to one of the tertiary analogue; abscissa, point of attachment to the pyridine ring. A negative value indicates that activity is increased by quaternization.

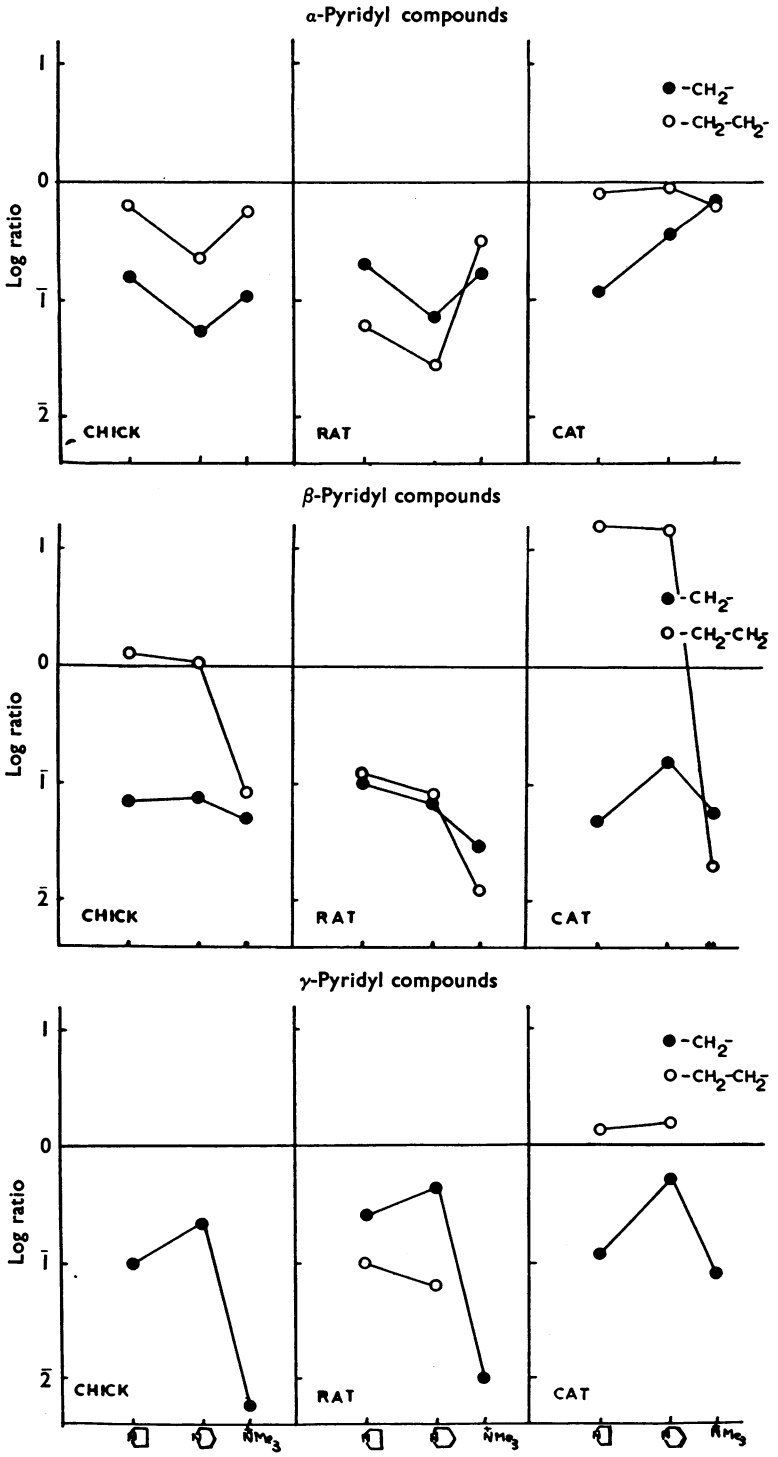


Fig. 5. Effect on activity of alteration of the side-chain amino-group. Ordinate, logarithm of the number of ions of the compound equivalent to one of the dimethylamino-analogue; abscissa, composition of the side-chain amino-group. A negative value indicates that the compound is more active than the dimethylamino-analogue.

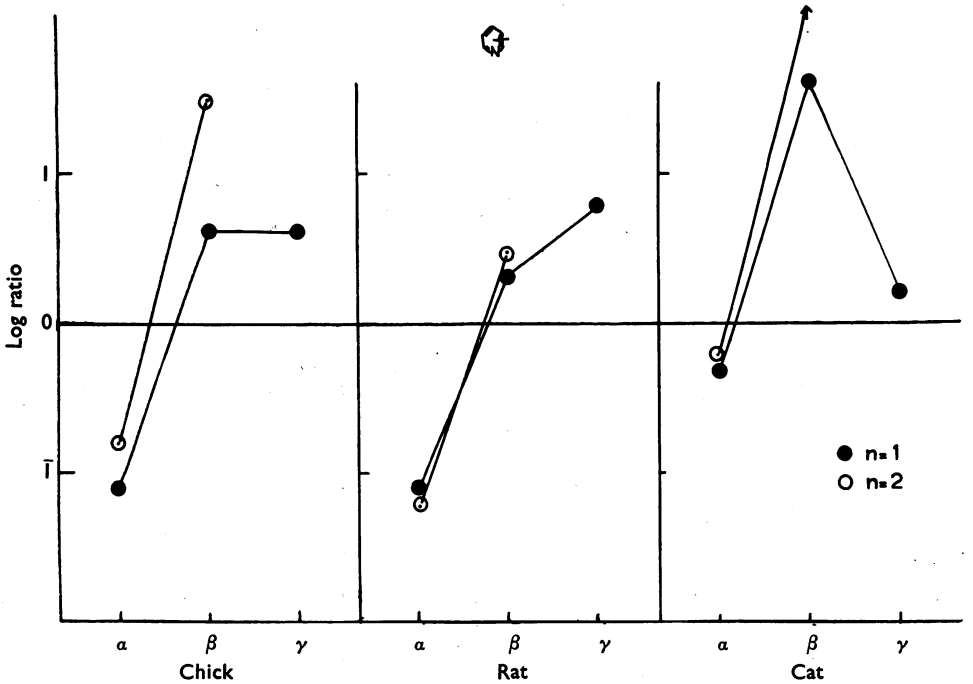


Fig. 6. Effect on activity of replacing the pyridyl group by a phenyl group. Ordinate, logarithm of the number of ions of the phenyl compound equivalent to one of the pyridyl analogue; abscissa, point of attachment to the pyridine ring. A negative value indicates that the phenyl compound is more active than the pyridyl analogue.

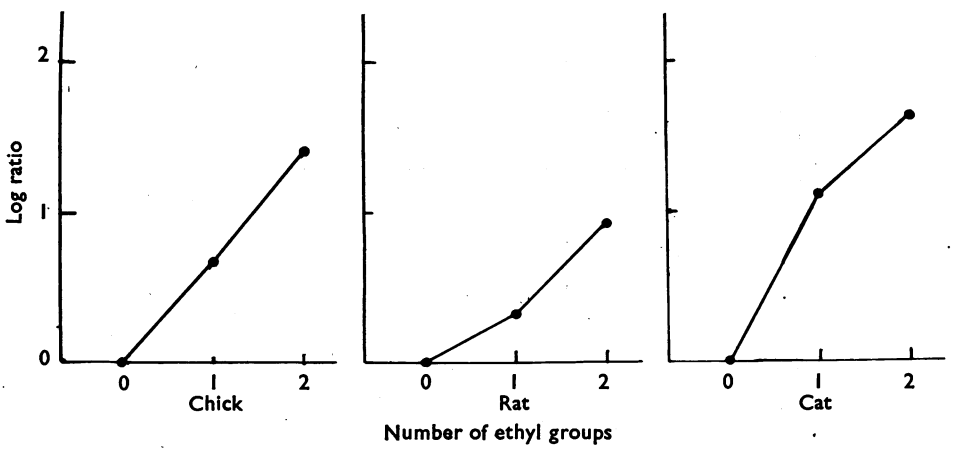


Fig. 7. Effect on activity of replacing methyl groups in trimethyl(3-pyridylmethyl)ammonium by ethyl groups. Ordinate, logarithm of the number of ions of the analogue equivalent to one of the trimethyl compound; abscissa, number of ethyl groups.

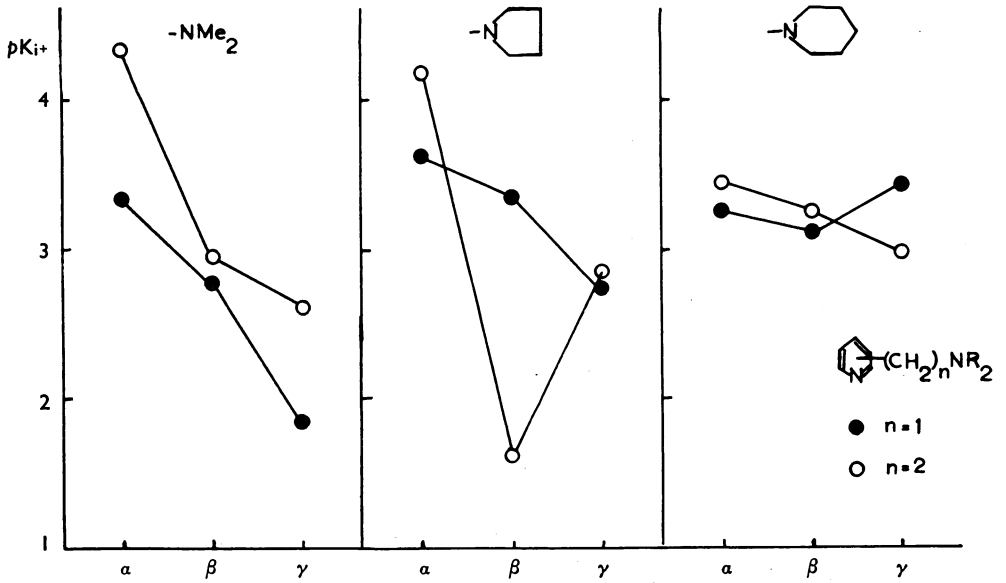


Fig. 8. Effect on anticholinesterase activity of point of attachment to the ring. Ordinate, logarithm of the reciprocal of the inhibitor constant expressed in terms of the concentration of univalent ion; abscissa, point of attachment to the pyridine ring. A high value here indicates a high activity.

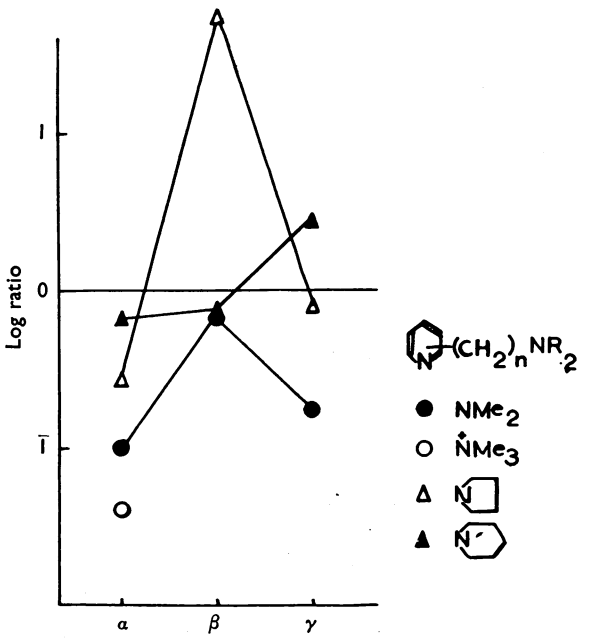


Fig. 9. Effect on anticholinesterase activity of chain length. Ordinate, logarithm of the number of ions of the ethylene derivative equivalent to one of the methylene analogue; abscissa, point of attachment to the pyridine ring. A negative value indicates that activity is increased by lengthening the chain.

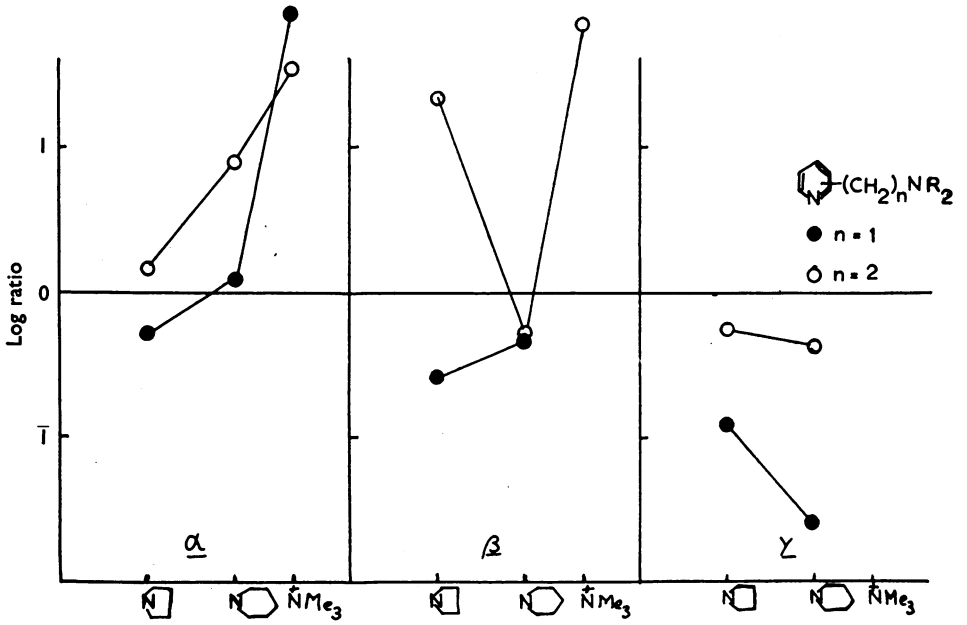
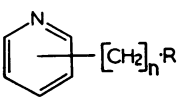


Fig. 10. Effect on anticholinesterase activity of alteration of the side-chain amino-group. Ordinate, logarithm of the number of ions of the compound equivalent to one of the dimethylamino-analogue; abscissa, composition of the side-chain amino-group. A negative value indicates that the compound is more active than the dimethylamino-analogue.

TABLE 5

EFFECT ON ANTICHOLINESTERASE ACTIVITY OF QUATERNIZATION OF THE SIDE-CHAIN AMINO-GROUP

Equipotent ionic ratios. A value greater than unity indicates decreased activity. Numerals indicate the number of ions of the quaternary metho-compound equivalent to one of the tertiary compound



Point of attachment	n=	R=	Compound no.	
α	1	Dimethylamino	1	85
α	2	Dimethylamino	4	35
β	1	Dimethylamino	5	69
β	1	Pyrrolidin-1-yl	8	1·6
	Nicotine			1·0

increasing substitution is quite different from that observed with analogues of hexamethonium (Wien, Mason, Edge & Langston, 1952), nor does it greatly resemble that observed with acetylcholine (Holton & Ing, 1949), in which there was a marked decline in activity when the second methyl group was replaced by an ethyl group.

Effects on anticholinesterase activity of various chemical features are illustrated in Figs. 8, 9, and 10 and Table 5. In the dimethylamino-series the α-compounds are more active than the β- or γ-compounds, and lengthening the side-chain increases the activity. In the pyrrolidine and piperidine series this is also true in general, but there are some exceptions, the inactivity of 1-[2-(3-pyridyl)ethyl]pyrrolidine (11)

being the most striking. Quaternization markedly reduces activity in the dimethylamino-series but not in the pyrrolidine series or with nicotine itself.

The most active compounds, dimethyl[2-(2-pyridyl)ethyl]amine (4) and 1-[2-(2-pyridyl)ethyl]pyrrolidine (10), are, however, only feeble anticholinesterases when compared with substances such as eserine, which are of the order of 1,000 times as active.

DISCUSSION

Information obtained from the tests. It was hoped that the test with the chick biventer would give an indication of the ability of the compounds to depolarize the neuromuscular junction and that this information could be extended to the ganglion. The ability to stimulate the ganglion was not systematically studied for two reasons. First, because the concentrations producing block were often close to those producing stimulation with the result that the dose-response curve tended to become flat at high levels and quantitative estimations of activity were difficult. Secondly, on the occasions when stimulant activity was estimated the results did not differ greatly from the estimates of blocking activity (see Table 3). There remains the possibility that some of the compounds produce a block of transmission in the ganglion by a different mechanism from that of nicotine. This is almost certainly true for the phenyl ether of homocholine (no stimulant activity ; equiblocking ionic ratio, 4.2) but not necessarily for the phenyl ether of choline (equistimulant ionic ratio, 0.25 ; equiblocking ionic ratio, 0.68). It would seem also to be likely to be true for the α- and γ-compounds listed on page 522, whose effects are much slower in onset than those of nicotine. With all the more active compounds, however, except homocholine phenyl ether, it was observed that in the experiments to test blocking activity the addition of the drug invariably caused a slight increase in the contracture of the nictitating membrane immediately before the block. It seems, therefore, likely that the results really do give some indication of nicotine-like activity on the ganglion.

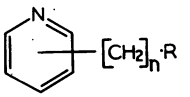
The results with the rat diaphragm preparation are likewise complicated by the possibility of producing a block by at least two different mechanisms, by an action like that of tubocurarine or by an action like that of decamethonium or of excess acetylcholine. On this preparation, perhaps even more than on the superior cervical ganglion, it is difficult to know how a particular compound is acting, but the observation that 1-[2-(4-pyridyl)ethyl]piperidine (18), 1-[2-(4-pyridyl)ethyl]pyrrolidine (12), dimethyl(2- and 4-pyridylmethyl)amine (1, 3) and dimethyl[2-(4-pyridyl)ethyl]amine (6) do not have appreciable activity on the chick biventer may be taken to indicate that their action on the rat diaphragm is likely to be like that of tubocurarine. It may be noteworthy that, although 1-[2-(4-pyridyl)ethyl]piperidine (18), for instance, is quite active on the rat diaphragm, it and the other compounds listed above are only very feebly active on the ganglion.

Another factor which may complicate the results is the anticholinesterase activity of the compounds. Although this is, in general, feeble, a comparison of the concentrations used in the *in vitro* experiments with the pK_i of the compounds for the acetylcholinesterase of dog caudate nucleus (Table 6) suggests that it might be affecting some of the results. Anticholinesterase activity would assist in producing

TABLE 6

COMPARISON OF THE CONCENTRATIONS EFFECTIVE ON THE VARIOUS PREPARATIONS

EC_{50} indicates the order of magnitude of the concentration effective on the chick biventer or rat diaphragm. ED_{50} indicates the order of magnitude of the concentration of which 0·1 ml. produces an effect on the ganglion. K_i is the inhibitor constant for the cholinesterase of the dog caudate nucleus



Point of attachment	n=	R=	Compound no.	Rat $-\log EC_{50}$	Chick $-\log EC_{50}$	Cat $-\log ED_{50}$	Cholinesterase $-\log K_i$
α	1	Dimethylamino	1	2·12	2·78	0·76	3·23
β	1	Dimethylamino	2	2·77	4·16	1·60	2·64
γ	1	Dimethylamino	3	2·65	3·10	0·21	1·58
α	2	Dimethylamino	4	2·49	3·60	0·99	4·32
β	2	Dimethylamino	5	2·77	4·05	2·35	2·93
γ	2	Dimethylamino	6	2·35	2·57	0·44	2·58
α	1	Pyrrolidin-1-yl	7	2·88	3·65	0·78	3·57
β	1	Pyrrolidin-1-yl	8	3·83	3·37	2·97	3·29
γ	1	Pyrrolidin-1-yl	9	3·39	5·26	1·29	2·64
α	2	Pyrrolidin-1-yl	10	3·70	3·81	1·08	4·18
β	2	Pyrrolidin-1-yl	11	3·69	3·94	1·10	1·61
γ	2	Pyrrolidin-1-yl	12	3·37	2·00	0·30	2·85
α	1	Piperidino	13	3·33	4·10	1·27	3·21
β	1	Piperidino	14	3·99	5·35	2·51	3·04
γ	1	Piperidino	15	3·08	3·85	0·57	3·26
α	2	Piperidino	16	4·06	4·26	1·04	3·44
β	2	Piperidino	17	3·88	4·07	1·12	3·22
γ	2	Piperidino	18	3·54	1·83	0·26	2·97
α	1	Trimethylammonium	19	2·99	3·86	1·01	1·41
β	1	Trimethylammonium	20	4·43	5·60	2·95	—
γ	1	Trimethylammonium	21	4·89	5·60	1·58	—
α	2	Trimethylammonium	22	2·99	3·86	1·20	2·81
β	2	Trimethylammonium	23	4·70	6·15	4·03	1·11
β	1	Ethyldimethylammonium	24	4·12	4·94	1·84	—
β	1	Diethylmethylammonium	25	3·51	4·21	1·62	—
β	1	1-Methylpyrrolidinium	26	4·82	5·43	2·37	3·16
β	1	1-Methylpiperidinium	27	3·87	4·21	0·81	—
		Monomethylnicotinium		4·66	5·54	2·18	2·19
		Nicotine		3·69	5·60	2·81	3·18
		Benzyltrimethylammonium	28	4·11	4·98	1·35	—
		Trimethylphenethylammonium	29	4·21	4·66	1·67	—

contracture on the chick biventer and in producing a desensitization type of block in the rat diaphragm and superior cervical ganglion tests, whereas it would decrease a competitive type of block. From the comparison it would seem unlikely that any of the results on the chick biventer are seriously likely to be affected as a result of inhibition of cholinesterase. The compounds in which the concentration of drug in the bath exceeds the value of K_i by the greatest amount are 1-[2-(4-pyridyl)ethyl]-piperidine (18) and 1-[2-(4-pyridyl)ethyl]pyrrolidine (12), and these in fact are inactive on the chick biventer. On the rat diaphragm the concentrations used are greater than on the chick biventer, and the anticholinesterase effects might be expected to upset the estimates of the activity of a number of compounds. With the exception of 1-[2-(2-pyridyl)ethyl]pyrrolidine (10) and 1-(2-pyridylmethyl)-pyrrolidine (7), the compounds whose activity is most likely to be affected are those, such as dimethyl(2-pyridylmethyl)amine (1), which are only very feebly active.

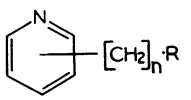
In the experiments with the ganglion the situation is complicated by the fact that the concentration of drug in the ganglion is not known. The concentration in Table 6 shown as EC_{50} is that which produced roughly 50% block when 0.1 ml. is injected. A comparison of the values of pK_i–log EC_{50} (Table 7) shows that for all the β-compounds, except 1-[2-(3-pyridyl)ethyl]piperidine (17), this value is less

TABLE 7

RATIOS OF CONCENTRATIONS AFFECTING PHARMACOLOGICAL TEST PREPARATIONS AND ENZYME

$$\log EC_{50} - \log K_i, \text{ i.e., } \log \frac{EC_{50}}{K_i}$$

When EC_{50} exceeds K_i, the log of the ratio is positive. The values for the cat superior cervical ganglion are for the ED_{50} shown in Table 6



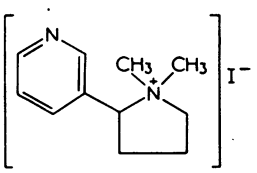
Point of attachment	n=	R=	Compound no.	Rat	Chick	Cat
α	1	Dimethylamino	1	+1·11	+0·45	+2·47
β	1	Dimethylamino	2	−0·13	−1·52	+1·04
γ	1	Dimethylamino	3	−1·07	−1·52	+1·37
α	2	Dimethylamino	4	+1·83	+0·72	+3·33
β	2	Dimethylamino	5	+0·16	−1·12	+0·58
γ	2	Dimethylamino	6	+0·13	+0·01	+2·14
α	1	Pyrrolidin-1-yl	7	+0·69	−0·08	+2·79
β	1	Pyrrolidin-1-yl	8	−0·54	−2·08	+0·32
γ	1	Pyrrolidin-1-yl	9	−0·75	−2·62	+1·35
α	2	Pyrrolidin-1-yl	10	+0·48	+0·37	+3·10
β	2	Pyrrolidin-1-yl	11	−2·08	−2·33	+0·51
γ	2	Pyrrolidin-1-yl	12	−0·52	+0·85	+2·55
α	1	Piperidino	13	−0·12	−0·89	+1·94
β	1	Piperidino	14	−0·95	−2·31	+0·53
γ	1	Piperidino	15	+0·18	−0·59	+2·69
α	2	Piperidino	16	−0·62	−0·82	+2·40
β	2	Piperidino	17	−0·66	+0·75	+2·10
γ	2	Piperidino	18	−0·57	+1·14	+2·71
α	1	Trimethylammonium	19	−1·58	−2·45	+0·40
α	2	Trimethylammonium	22	−0·18	−1·05	+1·61
β	2	Trimethylammonium	23	−2·59	−5·04	−2·92
β	1	Methylpyrrolidinium	26	−1·66	−2·27	−0·79
		Monomethylnicotinium		−1·47	−2·35	+1·01
		Nicotine		−0·88	−2·42	+0·37

than 1.5. It is only with α-compounds (the most active anticholinesterases) and the γ-compounds (which are only feebly active on the ganglion) that the value rises above 2.0. Prolonged blocking effects were only observed with the α-compounds, or γ-compounds already listed, and it seems reasonable to suppose that the results for the other compounds are not seriously modified by any inhibition of cholinesterase. This would imply that the drug concentration at the receptors in the ganglion is 1/100th of the concentration injected, which seems to be a dilution of a not impossible order of magnitude.

Relationships between structure and activity: chemical considerations. Before attempting to correlate activity in the various tests with the chemical structure of the compounds it is necessary to consider what information can be obtained from the latter which might be relevant to biological activity.

The distribution of the electrons in the pyridine ring, cited in Fig. 1 (which shows the relative net charge differences), will be disturbed by the various substituents. These, however, do not form a conjugated system with the pyridine ring, so, although it is not possible to compute their effects, these should not be so great as to alter the charge pattern radically. This is especially true in the 2-(pyridyl)ethyl compounds, where there are two carbon atoms between the side-chain nitrogen atom and the pyridine ring.

Other important information may be obtained by considering the arrangement of the atoms of the substances in space. The pyridylmethyl derivatives are remarkably rigid structures in which the distance between the side-chain nitrogen atom and the various atoms in the pyridine ring can vary only slightly. Rotation is possible about the bonds pyridine-C-N, but in the quaternary compounds this rotation is apparently restricted (judging from Courtauld or Catalin atomic models) by interaction between the substituents on the nitrogen atom and the pyridine ring. In nicotine monomethiodide (monomethylnicotinium iodide) (VI), for instance,



VI

this effect is so marked that it might be expected that this substance could exist in two distinct forms depending on the rotation of the pyridine ring. Fig. 11 shows the two arrangements for *S*-nicotine monomethiodide. In the pyridylmethyl compounds studied in this work it seems likely that the potential onium nitrogen atom is more or less fixed at an angle to the plane of the ring as indicated in Fig. 12. In the 2-(pyridyl)ethyl compounds, however, rotation is possible also about a third bond, resulting in a greater variety of movement. The potential onium nitrogen atom in

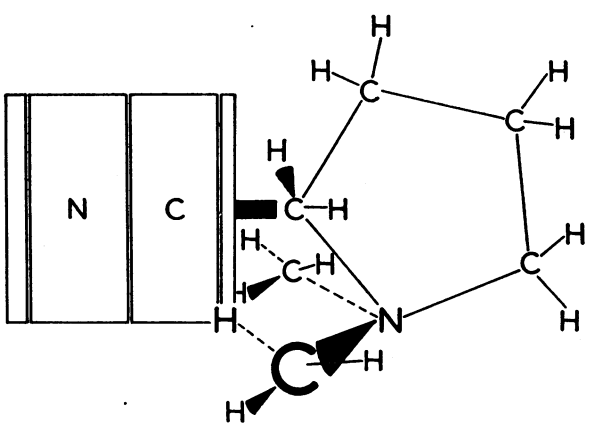


Fig. 11. Steric hindrance in *S*-nicotine monomethiodide. The pyridine ring is locked in place by the two methyl groups and a second form may exist in which the pyridine nitrogen atom is *trans* to the pyrrolidine nitrogen atom instead of *cis* as drawn here.

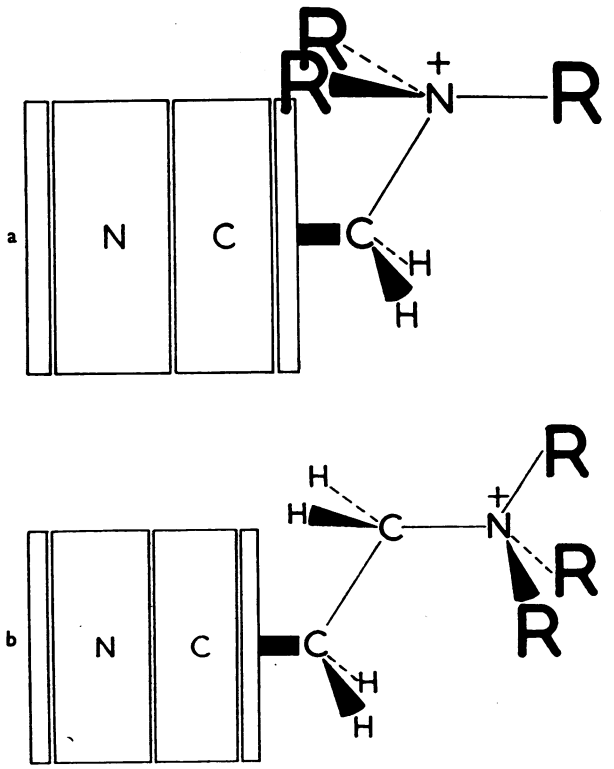


Fig. 12. (*a*) Pyridylmethyl compounds: rotation is only possible about two bonds and is restricted by the interaction of substituents R and the pyridine ring. (*b*) 2-(Pyridyl)ethyl compounds; rotation is possible about three bonds and there is less restriction because the larger substituents are further removed from the ring.

these molecules, for instance, could assume a position almost in the same plane as the pyridine ring.

Because the distances between the charged parts of the molecule must be important in determining biological activity, it is worth while considering what these may be in the various isomers. The figures in Table 8 are based on measurements from Courtauld atomic models and can obviously only be approximate. Nevertheless in the pyridylmethyl series, where there is rotation only about two bonds (and this is restricted for steric reasons in many compounds), these figures cannot be wholly uninformative. In the 2-(pyridyl)ethyl series, where more variation is possible, they may not be so meaningful.

It may also be important to note a difference between pyrrolidine and piperidine derivatives, particularly when these are quaternary. Because of the greater bulk of the piperidine ring (even though buckled and strainless) steric hindrance is likely to be greater in these compounds than in pyrrolidine derivatives (this could account for the difficulties experienced in the preparation of 1-methyl-1-(3-pyridylmethyl)-piperidinium bromide.

TABLE 8

POSSIBLE DISTANCES (Å) BETWEEN ONIUM ATOM IN (PYRIDYLALKYL)TRIMETHYLAMMONIUM SALTS AND OTHER ATOMS IN THE PYRIDINE RING

Point of attachment	Side-chain	Atoms in pyridine ring			
		2-C	4-C	6-C	N
α	$-CH_2-{}^+N(CH_3)_3$	2·0–2·2	4·2–4·6	3·7–4·8	2·4–3·8
β		2·5–3·4	2·5–3·4	4·6–4·7	4·1–4·7
γ		4·2–4·6	2·5–3·4	4·6–4·7	4·1–4·7
α	$-CH_2-CH_2-{}^+N(CH_3)_3$	2·0–4·0	4·4–5·4	4·1–5·9	2·7–4·5
β		2·5–4·6	2·5–4·6	5·0–6·5	4·6–5·3
γ		4·6–6·3	2·2–4·0	4·6–6·3	5·0–6·5
Nicotine		2·5–3·7	2·5–3·7	4·4–5·1	3·6–4·7
Monomethylnicotinium		2·4–2·6	2·8–3·1	4·4–4·5	3·8–3·9

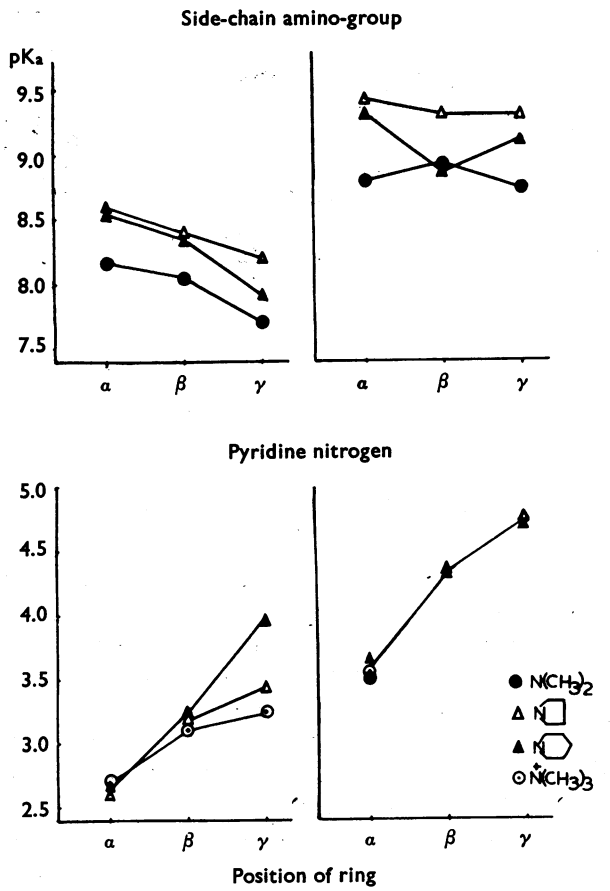


Fig. 13. Dissociation constants. Ordinate, pK_a; abscissa, point of attachment to the ring. Values for the side-chain amino-group are shown above, those for the pyridine nitrogen atom below; values for the pyridylmethyl series are shown on the left, those for the 2-(pyridyl)ethyl series on the right. Temperature, 25° C.

Variation of pK_a with structure. The effect of the position of the basic side-chain on the dissociation of the pyridinium proton (Fig. 13, Table 4) is what would be expected (see, for example, Albert, 1960). The positive charge on the much more strongly basic side-chain amino-group will tend to expel the pyridinium proton, and the strength of this effect will depend upon the distance between the two charged groups. The pK_a of the pyridine nitrogen atom will therefore increase in order depending upon whether the side-chain is in the α-, β-, γ-position, and the compounds with the ethylene side-chain will be stronger bases than those with the methylene side-chain. This explanation is supported by the results with the compounds containing a quaternary trimethylammonium side-chain, which show exactly the same variation of pK_a (for the pyridine nitrogen atom) and the point of attachment of the side-chain to the ring as do the analogous tertiary amines. The only aberrant result appears to be that for 1-(4-pyridylmethyl)piperidine (15), which is higher than would be expected.

The dissociation of the side-chain amino-group of the pyridylmethyl compounds shows a slight increase in pK_a in the opposite order, that is, depending on whether the side-chain is in the γ-, β-, α-position. This effect is not so marked in the 2-(pyridyl)ethyl compounds and might be ascribed to the influence of the partial negative charge on the pyridine nitrogen atom, which would tend to attract protons and hence strengthen the basicity of substances in which the side-chain amino-group is located close to this pyridine nitrogen atom. The effect of the substituents on the strength of the amino-group (pyrrolidin-1-yl slightly stronger than piperidino stronger than dimethylamino) is consistent with the values for the parent bases (pyrrolidine, 11.3; piperidine, 11.2; dimethylamine, 10.9; 1-methylpyrrolidine, 10.4; 1-methylpiperidine, 10.1; trimethylamine, 9.8; Albert, 1960), but the low basicity of 1-(3-pyridylmethyl)piperidine (14) is not. It is tempting to dismiss this result and the high basicity of the pyridine nitrogen atom in 1-(4-pyridylmethyl)piperidine (15) as being due to experimental error, but it is difficult to justify this because the experiments were done in duplicate and the other pK_a values of the substances concerned are consistent with the results with the other compounds (which they should not be if the trouble were due to impurities).

Inhibition of acetylcholinesterase. According to current theories (review by Davies & Green, 1958), the hydrolysis of acetylcholine by acetylcholinesterase involves binding of the substrate at two points termed the anionic site and the esteratic site. At the former there is electrostatic attraction between the positive quaternary nitrogen atom and a negative group on the enzyme. At the latter it is supposed that the carbonyl carbon atom (partially positive) interacts with the iminazole nitrogen atom in a histidine residue and the carbonyl oxygen atom (partially negative) forms a hydrogen bond with the hydroxyl group of a serine residue.

The possible points of attachment of the compounds tested in this work are the charged side-chain amino-group, the partial positive charges on the carbon atoms in positions 2, 4 and 6 and the relatively larger negative charge on the pyridine nitrogen atom. Anticholinesterase activity is greatest in the 2-(2-pyridyl)-ethyl derivatives, and it is possible to explain this by supposing that the side-chain

amino-group is attached at the anionic site and that the pyridine nitrogen atom resembles the carbonyl oxygen atom in acetylcholine in forming a hydrogen bond with the serine residue in the esteratic site. If the attachment to any extent involved the partial positive charges in positions 2, 4 and 6, it would be expected that the 2-(3-pyridyl)ethyl compounds would be stronger inhibitors than the α-analogues because the arrangement of the charges in these β-compounds would more closely resemble that in acetylcholine. The idea of the attachment of pyridine derivatives through hydrogen bonds involving the pyridine nitrogen atom has been suggested for the reactivators pyridine-2-hydroxamic acid and pyrimidine-2-hydroxamic acid. If it applies here, dimethyl[2-(2-pyridyl)ethyl]amine (4) should be a much more potent anticholinesterase than dimethylphenethylamine.

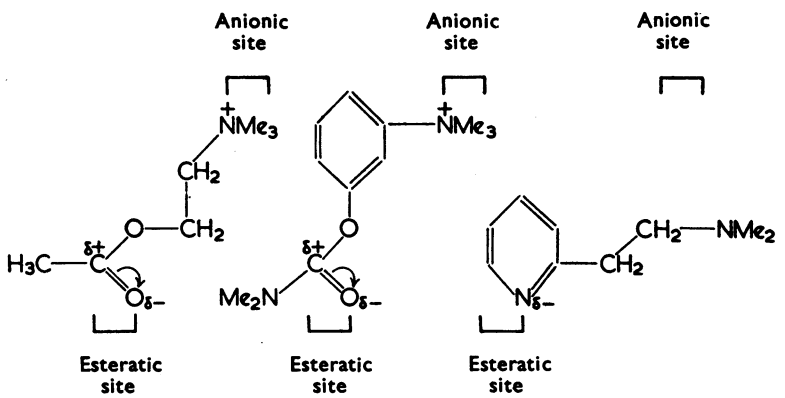


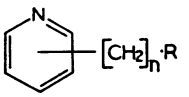
Fig. 14. Comparison of acetylcholine, neostigmine and dimethyl[2-(2-pyridyl)ethyl]amine, to show that in the latter the potential onium group is unlikely to occupy the same position relative to the esteratic site as it does in acetylcholine or in neostigmine.

The finding that quaternization reduces anticholinesterase activity is surprising, because by improving the binding at the anionic site it should increase activity. Neostigmine, for instance, is about 100 times as active as its tertiary analogue (Aeschlimann & Reinert, 1931). It can be taken as evidence that the binding of these pyridine derivatives is not analogous to that of acetylcholine or neostigmine, and supports the idea that attachment through the partially negative nitrogen atom in the ring is more important than attachment at any partially positively charged carbon atom (Fig. 14). It should be noted that quaternization of the (3-pyridylmethyl)dialkylamines does not affect anticholinesterase activity markedly.

Relationships between structure and activity in the pharmacological tests. For convenience the more active compounds are shown together in Table 9. If only the results for the pyridylmethyl compounds on the cat superior cervical ganglion are considered, these (Fig. 2a) fulfil the expectation that activity should be greatest in the β-isomers, in which the partial positive charge on the carbon atoms in positions 2 or 4 is a similar distance from the onium nitrogen atom as is the oxygen atom in the phenyl ethers of choline.

TABLE 9

ABBREVIATED RESULTS. EQUIPOTENT IONIC RATIOS



Chick					Cat				
Point of attachment	n=	R=	Compound no.		Point of attachment	n=	R=	Compound no.	
β	2	Trimethylammonium	23	0·39	β	2	Trimethylammonium	23	0·088
		Nicotine		1·0	β	1	Pyrrolidin-1-yl	8	0·84
β	1	Trimethylammonium	20	1·4	β	1	Trimethylammonium	20	0·98
γ	1	Trimethylammonium	21	1·4			Nicotine		1·0
		Monomethylnicotinium		1·7	β	1	Piperidino	14	2·8
β	1	Pyrrolidin-1-yl	8	2·0					
β	1	1-Methylpyrrolidinium	26	2·1					
					Rat				
					γ	1	Trimethylammonium	21	0·088
					β	1	1-Methylpyrrolidinium	26	0·10
					β	2	Trimethylammonium	23	0·14
							Monomethylnicotinium		0·15
					β	1	Trimethylammonium	20	0·25
							Trimethylphenethylammonium	29	0·42

The activity of trimethyl(3-pyridylmethyl)ammonium (2), however, is greatly increased by lengthening the side-chain, but this change in structure does not always lead to an increase in activity. In the dimethylamino-series there is some increase, especially with the β-isomer, but in the other series increasing the chain length decreases the activity. It is also striking that, although replacement of the dimethylamino-group by a pyrrolidin-1-yl or, to a lesser extent, by a piperidino group leads to increased activity in the pyridylmethyl series, it decreases activity in the pyridylethyl series. Further, although quaternization increases activity in the dimethylamino-compounds of either the pyridylmethyl or pyridylethyl series, it decreases the activity of the pyrrolidine or piperidine derivatives.

It seems probable that there may be a limit to the size of this part of the molecule which may be tolerated and that increasing the bulk leads to increased activity until the limit is reached. This, however, cannot be a complete explanation because it does not explain why ethyldimethyl(3-pyridylmethyl)ammonium (24) is much less active than its isomer trimethyl[2-(3-pyridyl)ethyl]ammonium (23).

If the activity is also influenced by the fit at a second point besides the onium nitrogen atom, involving a partial positive charge on the drug and a negative receptor group, it should be possible to obtain some idea of the position of this group, relative to the receptor group which binds the onium nitrogen atom, by comparing the distances in Table 8 with the activity of the compounds (Table 9). When the partially negative pyridine nitrogen atom lies between the onium nitrogen atom and the partially positive carbon atom, it is possible that, although the distance between the two latter may be the same as that between the two receptor groups, the compound will fail to be attached at the receptor because of repulsion between

the negative receptor group (binding the partially positive carbon atom) and the partially negative pyridine nitrogen atom. If this is assumed (it will apply to the α-substituted compounds), the results are consistent with the distance between the receptor groups in the ganglion being somewhere around 3.4 Å. Both the carbon atoms in positions 2 and 4 in the pyridine ring in trimethyl[2-(3-pyridyl)ethyl]-ammonium (23) should fit well, the 3-pyridylmethyl derivatives and also nicotine to a lesser extent, but the α- or γ-compounds will be a poor fit.

It must be emphasized that the situation is complicated, because activity on this preparation, whether stimulant or blocking, depends on two properties, ability to depolarize and ability to desensitize, which may not be connected and which act in opposition when producing the pharmacological response. On the chick biventer, however, the situation is simpler, because the test is only concerned with ability to depolarize. There is nevertheless a general similarity between the results in this test and those on the cat ganglion. As with the latter, if only the pyridyl-methyldialkylamines are considered the expectation that activity should be greatest in the β-isomers is fulfilled. The replacement of the dimethylamino-group by a pyrrolidin-1-yl or piperidino-group increases activity in the pyridylmethyl series and decreases activity in the 2-(pyridyl)ethyl series: lengthening the side-chain only increases activity in the β-substituted trimethylammonium compound (and in the α-substituted dimethylamino-compound): quaternization only increases the activity of the dimethylamino-compounds. The activity of trimethyl(4-pyridylmethyl)-ammonium (21), however, is unexpectedly high, being equal to that of the β-isomer, and it is also remarkable that nicotine itself is very active (except for trimethyl-[2-(3-pyridyl)ethyl]ammonium (23) it is the most active compound).

These results suggest that, although the receptor group binding the onium nitrogen atom is very similar to that in the ganglion, the distance between the two receptor groups is not the same. It seems possible that they may be slightly further apart. A distance of 3.7 Å would allow a good fit by trimethyl[2-(3-pyridyl)ethyl]ammonium (23) and, to a lesser extent, by nicotine. The distance in trimethyl(3-pyridylmethyl)-ammonium (20) would normally be too short and in trimethyl(4-pyridylmethyl)-ammonium (21) too long for a good fit, and the fit of the α-isomer (which might be attached at the carbon atom at position 6) would be disturbed by the proximity of the partially negative pyridine nitrogen atom in between the carbon atom at position 6 and the side-chain nitrogen atom.

The results with the rat diaphragm are rather different, even though the pharmacological process involved—desensitization—should be at least the same as leads to block in the ganglion. There seems to be an increase in the size of the groups which may advantageously be attached to the side-chain nitrogen atom. The replacement of the dimethylamino-group by a pyrrolidin-1-yl or piperidino-group leads to an increase in activity in both the pyridylmethyl and 2-(pyridyl)ethyl series: increasing the length of the side-chain does not lead to such a decline in activity as in the other tests: quaternization increases activity in all the instances studied except 1-(3-pyridylmethyl)piperidine (14) (and even with this compound the decline in activity on quaternization is not as marked as in the other tests). It is also striking that in this test many compounds are more active than nicotine; nicotine

monomethiodide, for instance, is over 6 times as active as the tertiary base. Yet another contrast is provided by the smaller differences between the activities of the α-, β-, and γ-isomers—the expectation of highest activity in the β-isomer is not convincingly shown—and, in particular, by the extremely high activity of trimethyl-(4-pyridylmethyl)ammonium (21).

These results can be taken to indicate a much greater distance between the two receptor groups than in either the chick biventer or the cat ganglion. If the distance were around 4.5 Å, trimethyl(4-pyridylmethyl)ammonium (21) should fit well, trimethyl[2-(3-pyridyl)ethyl]ammonium (23) should fit comparably, 3-pyridylmethyl compounds less well and even 2-pyridylmethyl compounds to some extent. The 2-(2- and 4-pyridyl)ethyl compounds should also fit, and the order of activity would depend upon this ability to fit the two receptor groups and the influence of the group attached to the side-chain nitrogen atom on the fit of this onium group.

Although there are, quite logically and properly, many objections to this kind of explanation of differences between activity, many of the compounds studied in this work are isomers, and it is difficult to see how else to account for differences in pharmacological activity observed in tests which have been designed to depend, as far as possible, only on one particular action. If the explanations are accepted, at least as a working hypothesis, they may give some clue as to the binding of acetylcholine at these sites. Sörum (1959) has shown that, in the crystal, acetylcholine exists in two forms, a " ring " form and an " extended " form. Although in the latter the distance between the carbonyl carbon atom and the onium nitrogen atom is around 4.5–5 Å, in the ring form it is very much less. The distances cited in this discussion, therefore (3.4–4.5 Å), are not inconsistent with the hypothesis that in acetylcholine the partially positive carbonyl carbon atom of the ester link is a more important pharmacodynamic group than the ether oxygen atom which, in acetylcholine (but not in choline phenyl ether), should not carry a positive change of any size.

There is a striking resemblance between trimethyl[2-(3-pyridyl)ethyl]ammonium (23) and leptodactyline [*m*-hydroxyphenethyl)trimethylammonium ; Glässer, 1960 ; Erspamer & Glässer, 1960]. From the estimate—eight times as active as nicotine—for the activity of leptodactyline on the cat superior cervical ganglion, and from the concentration (7–15 μg/ml., that is, approximately 2×10^{-5} M) used on the rat diaphragm preparation, it seems that the compounds should have comparable activity. It is, therefore, particularly interesting that Glässer & Pasini (1960) have found that replacement of the hydroxyl group by a methoxyl group, and, to a greater extent, by a methyl group, lowers activity.

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