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ON RELATIONSHIPS BETWEEN STRUCTURE AND NICOTINE-LIKE STIMULANT ACTIVITY IN CHOLINE ESTERS AND ETHERS

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Quaternary nitrogen compounds and their analogues, collectively designated as " onium " compounds, exhibit a considerable variety of pharmacological actions, and the study of structure-action relationships in this group has been a fruitful one for pharmacologists since the original and classical observations of Crum Brown and Fraser (1869).

A large number of " onium " compounds have the property of stimulating autonomic ganglion cells when injected into the circulation. This property was first demonstrated for the non-quaternary compound nicotine and is generally referred to as a nicotine-like stimulant effect.

The extensive studies of Hunt and Taveau (1911) enabled them to make the generalization that maximum activity (both " muscarinic " and " nicotinic ") was to be found in compounds containing the grouping—

$$—O—CH_2CH_2\overset{+}{N}(CH_3)_3$$

Much subsequent work in the field by Hunt and his co-workers and by many others has tended to confirm this early generalization.

An examination of this work led the author (1949) to extend this early observation, and to put forward a tentative hypothesis that maximum nicotine-like stimulant action would be found in ions of the type, $RO.CH_2CH_2\overset{+}{N}(CH_3)_3$, where the character of the group R is such that maximum mesomeric deviation towards structures of the type—

$$\bar{R}: \overset{+}{O}.CH_2CH_2\overset{+}{N}(CH_3)_3$$

would be expected.

The first part of this paper is devoted to a review of the literature on compounds with nicotine-like stimulant activity, and presents the evidence which led to the formulation of the hypothesis. It is not practicable to include all available data, and only such points as appear pertinent to the argument will be brought forward. The second part of the paper provides evidence in support of the hypothesis based on a quantitative study of the activities of some of the compounds mentioned in the first part, and also of some new ones prepared in view of their theoretical interest.

PART I

A very large number of " onium " ions, exhibiting very diverse structures, are capable of stimulating autonomic ganglia. Of these, the great majority only do so when the dose is so large as to make it undesirable to attribute any great pharmacological significance to the results. A careful search confirmed the generalization that, with the exception of nicotine itself, all ions showing a high degree of activity* contain the structure—

$$-O-C-\overset{+}{C}-N(CH_3)_3$$

A large number of compounds containing this structural unit have been made, and their pharmacological properties investigated. Compounds in this category, however, include substances with the highest degree of activity so far described, and others which appear to be completely inactive.

The evidence from the literature in favour of specific structural requirements will be dealt with under three headings.

(i) *The structure of the cationic head of the ion*

Both muscarine-like and nicotine-like stimulating compounds appear to require that three methyl groups be attached to nitrogen if maximum activity is to be preserved. Replacement of the methyl groups by larger alkyl groups or heterocyclic rings always reduces activity. If the " onium " nitrogen atom is replaced by phosphorus, arsenic, antimony, or sulphur the activity is likewise reduced.

Burn and Dale (1914) showed that whereas tetramethylammonium has pronounced activity, tetraethylammonium is inactive and, in fact, has a blocking action. Acheson and Moe (1946) and Acheson and Pereira (1946) have shown that tetraethylammonium has a weak stimulating action, although it is certainly very much less pronounced than with tetramethylammonium.

Replacement of one methyl group of tetramethylammonium by *n*-butyl, benzyl, phenyl, vinyl, or allyl groups was shown (Hunt, 1926) to give compounds which were all less active than the parent ion. Among other simple quaternary compounds Hunt and Renshaw (1925) found that tetramethylphosphonium, tetramethylarsonium, tetramethylstibonium, and trimethylsulphonium were all less active than tetramethylammonium.

Holton and Ing (1949) studied the effects of successively replacing the methyl groups by ethyl in the cationic head of acetylcholine, and observed a rapid reduction of both muscarine-like and nicotine-like stimulant activities as each change was made.

Choline phenyl ether (Hunt and Renshaw, 1929a) appears to have the greatest activity, with the possible exception of the phenyl ether of α-methylcholine (Hunt and Renshaw, 1936); but β-phenoxyethyltriethyl ammonium bromide (Hunt and Renshaw, 1933 ; Bülbring and Depierre, 1949) is inactive. Various other β-phenoxyethyl compounds in which the " onium " nitrogen is part of heterocyclic structures show only very slight activity (Hunt and Renshaw, 1929b); β-phenoxyethyldimethylsulphonium iodide shows approximately one-tenth the activity of choline phenyl ether (Hunt and Renshaw, 1932), and Hunt and Renshaw (1925) stated that the phosphonium analogue of acetylcholine is inactive.

* In this and subsequent parts of the paper the word " activity " without other qualification should be taken to mean " nicotine-like stimulating activity."

If, in substances which otherwise show appreciable activity, methyl groups of the cationic head are replaced by hydrogen atoms a large fall in activity occurs ; this is probably to be associated with the reduced stability of the ion, since stability appears to be essential for high activity. Levy and Ditz (1934) studied the properties of β-phenoxyethylamine and N–(β-phenoxyethyl) dimethylamine, which represent the application of this process to the very active choline phenyl ether ; they found both these compounds to have a weak pressor effect, but they did not determine whether this was a nicotinic action. The second of these compounds was, however, found by Justin-Besançon and Kohler (1937) to stimulate autonomic ganglia ; from their evidence the writer estimates the activity to be of the order of one five-hundredth or less that of choline phenyl ether. Stehle, Melville, and Oldham (1936) applied the same process to acetylcholine and were unable to demonstrate nicotine-like actions in the compounds produced.

These observations confirm generalizations made by previous workers that the cationic head of the ion must be $-\overset{+}{N}(CH_3)_3$ if maximum activity is to be preserved.

(ii) *Influence of the chain structure interposed between the "ether" oxygen and quaternary nitrogen atoms*

The structure of the chain interposed between the "ether" oxygen and the quaternary nitrogen atom appears to be highly critical, and must be two carbon atoms if the ion is to show a high degree of activity.

The critical value of chain length may be illustrated by the activities of many compounds described in the literature, and, in all these examples, the derivatives of "γ-homocholine" and "formocholine" are much less active than the corresponding choline derivatives ; thus, although choline phenyl ether has an intense nicotine-like action, the phenyl ether of γ-homocholine has been shown (Hunt and Renshaw, 1929c) to have negligible activity. The same relationship applies in the benzoyl esters of choline and γ-homocholine ; the former (Hunt and Taveau, 1906) appears to be feebly nicotine-like, while the latter is very much less active. Acetyl-γ-homocholine (Hunt and Taveau, 1911) is also less active than acetylcholine.

Data for compounds with a shorter chain length are of doubtful value for, as Stewart and Kung (1933) have pointed out, such compounds are extremely unstable, and thus proof of structure is lacking. Hunt and Renshaw (1925), however, reported that acetylformocholine was less active than acetylcholine.

One exception to this rule was reported by Simonart (1928), who found the methyl ether of γ-homocholine to be more active than the corresponding choline derivative. Since, however, both these compounds show only a low order of activity it would not appear to be a serious objection to the argument.

Branching of the chain may profoundly modify the activity of choline derivatives. If the branch is at the α-carbon atom the activity does not appear to be greatly reduced, and may possibly be slightly increased, although too few examples of this modification have been described to permit generalization. Simonart (1932) reported that acetyl-α-methylcholine was as active as acetylcholine, while Hunt and Renshaw (1936) state that the phenyl ether of α-methylcholine has perhaps twice the activity of the phenyl ether of choline.

Beta-substitution appears to reduce activity greatly, although it has not been tried in compounds which otherwise show high degrees of activity. Hunt and Renshaw

(1936) mention the phenyl ether of β-methylcholine, but no account of its pharmacology has been given. It is well known that acetyl-β-methylcholine lacks the nicotine-like properties of acetylcholine (Hunt and Taveau, 1911 ; Simonart, 1932 ; Comroe and Starr, 1933).

Simonart (1932) also studied propionyl-β-methylcholine and found it less active than propionylcholine, and the same author (1934) reported that acetyl-β-ethylcholine is inactive. Simonart (1934) states that carbamyl-β-methylcholine has no nicotine-like activity although carbamylcholine is very active.

The alkyl ethers of choline and β-alkylcholines, extensively studied by Simonart (1932, 1934), show a few anomalies. Although alkyl ethers of choline do not, in general, exhibit appreciable activity it should be pointed out that the *n*-butyl ether of choline is slightly active. The methyl and ethyl ethers show negligible activity (Dale, 1914 ; Simonart, 1928, 1932), but Simonart (1934) ascribed weak nicotine-like properties to the methyl ether of β-butylcholine, although the corresponding ethyl ether was inactive. No other alkyl ethers of β-alkylcholines appear to have appreciable activity.

An apparent exception to this rule that β-substitution reduces activity is seen in a series of compounds examined by Renshaw, Dreisbach, Ziff, and Green (1938). These workers found that acetyl-β-methylthiocholine was more active than acetylthiocholine which, in turn, was more active than acetylcholine.

The compound Meprochol (2-methoxypropenyltrimethylammonium bromide) which, for reasons which will appear in the next section, would be expected to show some activity is, in fact, inactive (Hecht, 1935) ; this inactivity no doubt being due to the fact that it may properly be regarded as a β-substituted choline derivative.

Betaine and its esters show the same requirements, as already stated, of quaternary nitrogen to " ether " oxygen distance, although in these instances the β-carbon atom is part of a carbonyl group. These compounds (Hunt and Renshaw, 1926) show no, or relatively little, activity. Betaine amide and anilide, which lack the " ether " oxygen, may show greater activity than the esters, in which the " ether " oxygen is present (Hunt and Renshaw, 1926, 1929c).

Homologues of betaine derivatives have been described by Bass, Schueler, Featherstone, and Gross (1950) and Burgen and Hobbiger (1949) ; these compounds show some activity although not of a high order.

The actions of a number of cyclic compounds, which may be regarded as β-substituted choline derivatives, have been studied. The most active of these would seem to be compound 2249F, described by Fourneau, Bovet, Bovet, and Montézin (1944), although the work of Ambache (1949) makes it doubtful if its action is truly nicotinic. The lactone compound, δ-trimethylammonium-γ-valerolactone (759L) studied by Dallemagne and Philippot (1949) would appear to be moderately active in the dog. Furfuryltrimethylammonium and tetrahydrofurfuryltrimethylammonium bromides (Fellows and Livingston, 1941, 1942) are extremely weak and, even in doses of the order of 10 mg./kg., produce responses in only about half of the animals used.

(iii) *The influence of the group R attached to the " ether " oxygen atom*

The available evidence strongly suggests that the electron density of the ether oxygen atom is an important factor and, further, that activity increases as the electron

density decreases. Examples of this are readily found in the large number of choline ethers which have been described, and the more significant of these will be briefly mentioned.

Various observers (Dale, 1914; Burn and Dale, 1914; Simonart, 1928, 1932) have shown that choline ethyl ether has, at most, very feeble nicotine-like properties; but Simonart (1932) reported that the vinyl ether showed a substantially higher degree of activity. The spatial characteristics of these two molecules are essentially similar, but the fact that resonance can occur between the vinyl group and the oxygen atom, with consequent reduction of electron density of the oxygen, and that this cannot occur in the ethyl ether, suggests that this reduction may be associated with the increased activity.

A substantial mass of further evidence also tends to demonstrate this apparent connexion. As already stated, choline phenyl ether is extremely active, and this activity might be associated with the higher degree of resonance to be expected in this compound as compared with the vinyl ether. In the benzyl ether, where, by interposition of a $-CH_2-$ group between the phenyl group and oxygen, this resonance is diminished, we would expect a less active compound. Hunt and Renshaw (1936) have shown that a very marked reduction in activity does in fact occur when this modification is made.

Nuclear substituents could increase or decrease the activity relative to choline phenyl ether according to whether they operated to increase or decrease the electron density of the oxygen atom. In the first category no compounds have been noted, but in the second Hunt and Renshaw have described many and, in all instances, found reduced activity. These substituents, with references, are: 2-methyl, 3-methyl, 4-methyl, 4-ethyl, 4-*tert*-butyl, 2-*iso*propyl-5-methyl, 4-amino (1936); 2-methoxy, 4-methoxy, 4-hydroxy, 4-acetamido, 4-benzoyloxy (1929a).

Choline esters show more complex relationships than the ethers, and a wider variety of pharmacological actions. The benzilic ester (Ing, Dawes, and Wajda, 1945) and N : N-dialkylcarbamic esters (Swan and White, 1944) have marked atropine-like actions; the succinic ester (Bass *et al.*, 1950) has a powerful neuromuscular blocking effect; the nitric ester (Hunt and Renshaw, 1925) has a marked ganglion blocking action.

In esters of the type—

$$R-\overset{\overset{O}{\|}}{C}-O.CH_2CH_2\overset{+}{N}(CH_3)_3$$

resonance may take place either between the group R and the carbonyl group or between the carbonyl group and the ether oxygen atom. On the basis of the hypothesis proposed, activity should increase when the character of the group R is such that it enters less readily into resonance with the carbonyl group.

Although many choline esters have been studied the published results are not, in general, sufficiently quantitative to permit conclusions to be drawn concerning their relative activities. Some indication of the results which would be expected in this series, together with some of the actual results obtained by previous workers will, however, be considered.

If, in acetylcholine, one hydrogen of the acetyl group is replaced by successively longer alkyl chains, a sharp increase in activity would be expected in passing from acetyl to propionyl, followed by smaller increases as the chain is extended to give

the *n*-butyryl and *n*-valeryl esters. This series was the subject of a quantitative study by Simonart (1932) and Chang and Gaddum (1933), who found the activity to increase in the expected order, i.e., activity ratios for the acetyl, propionyl, butyryl, valeryl esters of approximately 1 : 2.5 : 5 : 5.

More striking increases should be observed in the series formed by replacing each hydrogen atom in the acetyl group, to yield the series acetyl-, propionyl-, *iso*-butyryl-, pivalyl- (trimethylacetyl-) cholines. All these have been studied (Hunt and Taveau, 1911 ; Simonart, 1932 ; Chang and Gaddum, 1933 ; Schweitzer, Weizmann, and Wright, 1938), but it is impossible to draw conclusions about their relative activities from the published results.

Benzoylcholine would also be expected to show considerably less activity than phenacetylcholine, and this was found to be so by Hunt and Taveau (1911).

Carbamylcholine, of all esters described, would appear to be anomalous, for the hypothesis would predict, in the absence of other determining factors, a low activity. Numerous observers have reported a high activity for this compound, the lowest estimates appearing to be those of Feldberg (1932) and Bender, Spirtes, and Sprinson (1943), who found the activity to be about one half that of acetylcholine. N-alkyl substitution would be expected to reduce the activity of carbamic esters, and this has been demonstrated to be so by Bender *et al.* (1943).

There is thus considerable evidence from the literature in favour of the hypothesis proposed. Further evidence, from a quantitative study of the activity of some of the compounds already mentioned and others, prepared for the purpose, will now be considered.

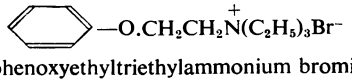
Part II

Part I of this paper indicated the desirability of securing quantitative evidence for the nicotine-like stimulant activity of a number of ethers and esters of choline, and certain related compounds, the order of activity of which should be predictable on the basis of the hypothesis there proposed.

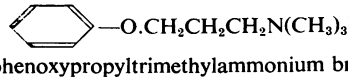
Of choline derivatives showing this kind of activity, choline phenyl ether was apparently the most active, and it was decided to study, under standard experimental conditions, the effects of—

(i) Modifications to the cationic head and the chain structure in this molecule.
(ii) Nuclear substituents.

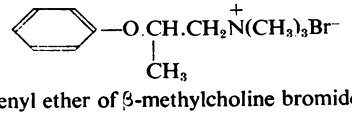
For the studies in the first category the following compounds were made to illustrate the effects of modifying the cationic head, of lengthening the chain, and of branching in the β position.



β-phenoxyethyltriethylammonium bromide.



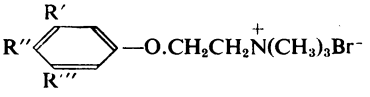
γ-phenoxypropyltrimethylammonium bromide.



phenyl ether of β-methylcholine bromide.

The pharmacology of two of these compounds has been previously described (see Part I for references), and the phenyl ether of β-methylcholine, although described by Hunt and Renshaw (1936), has not been tested pharmacologically.

In the second category a number of nuclear substituted phenyl ethers of choline had been studied by Hunt and his co-workers and all seemed to be less active than the unsubstituted compound, this diminution being, however, in accordance with the proposed hypothesis. It appeared, therefore, of considerable interest to ascertain whether an increase of activity could be obtained by suitable substitution in choline phenyl ethers. The choice of substituents likely to effect such a change is considerable, but many of these are relatively complex polar entities and thus might introduce factors which would obscure the argument. For this reason it was decided to make a quantitative study, under standardized conditions, of a series of nuclear substituted choline phenyl ethers in which the substituents were either methyl or halogen, and the following compounds, some of which are new, were prepared for the purpose :



Choline phenyl ether bromide (R′ = R″ = R‴ = H)

Choline *p*-chlorophenyl ether bromide (R″ = Cl; R′ = R‴ = H)

Choline *m*-chlorophenyl ether bromide (R‴ = Cl; R′ = R″ = H)

Choline *m*-bromophenyl ether bromide (R‴ = Br; R′ = R″ = H)

Choline 3 : 5-dibromophenyl ether bromide (R′ = R‴ = Br; R″ = H)

Choline *p*-tolyl ether bromide (R″ = CH_3; R′ = R‴ = H)

Choline *m*-tolyl ether bromide (R‴ = CH_3; R′ = R″ = H)

Choline 3 : 5-xylyl ether bromide (R′ = R‴ = CH_3; R″ = H)

The volumes occupied by the substituent groups in these molecules are approximately equal, the van der Waals diameters being approximately 3.0 A. for the methyl group, and 2.9 A. and 3.15 A. for chlorine and bromine respectively. This would make it unlikely that changes in activity were due to purely dimensional differences in the molecules.

If the hypothesis were tenable the phenyl, *m*-tolyl, 3 : 5-xylyl, and *p*-tolyl ethers of choline would be expected to show decreasing activity in that order, since in choline *m*-tolyl ether the inductive effect of the methyl group would operate to diminish the electron displacement postulated for maximum activity. The 3 : 5-xylyl ether having two such groups would show a further reduction in activity, while in the *p*-tolyl ether the inductive effect would be reinforced by the (hyperconjugative) + E effect and a large fall in activity would accordingly be expected.

In choline *m*-chlorophenyl ether, choline *m*-bromophenyl ether, and choline 3 : 5-dibromophenyl ether the inductive effects of the halogen atoms would tend to increase

the electron displacement and, in consequence, increased activity would be expected. The activity of choline *p*-chlorophenyl ether would not be readily predictable since, in this, the –I effect is opposed by the +E effect; it would, however, be expected to show greater activity than choline *p*-tolyl ether.

In addition to the above ethers a series of choline esters were chosen for study under similar conditions. These were, for reasons already outlined in Part I, acetylcholine, propionylcholine, *iso*butyrylcholine, pivalylcholine, benzoylcholine, phenacetylcholine, and carbamylcholine. The acetic, benzoic, and carbamic esters were commercial samples (as chlorides) while the remainder were iodides synthesized in this laboratory.

Nicotine, since it is the most familiar substance with this kind of action, was also included.

The changes in activity to be expected in this series have already been made clear in Part I.

Methods

Estimates of activity were made by comparing the pressor effects of the different compounds when given by intravenous injection to cats anaesthetized with chloralose and treated with at least 1 mg./kg. of atropine sulphate. Arterial pressures were recorded from a common carotid artery, and injections were made into the internal saphenous vein. Duplicate experiments were also done in animals in which the suprarenals were tied off in order to eliminate possible complications due to selective actions on these organs (Simonart, 1932). Although the use of spinal preparations would have been desirable, it was found that when the suprarenals were tied off the animals did not survive long enough to enable the necessary number of observations to be made.

In order to exclude the possibility that peripheral actions contributed to the pressor response it was confirmed, for each drug, that the response to an effective dose was abolished by known ganglion-blocking agents.

Choline phenyl ether was used throughout as the standard of comparison, since it was not only very active but also appeared to have negligible ganglion-blocking activity. In a few instances direct comparisons between other pairs of substances were made, and the results so obtained were fitted in by interpolation.

In making comparisons, suitable doses of two compounds were injected in random order so that, for each, points above and below a 60 mm. hypertension were obtained, this being about half the maximal response for animals under the conditions of the experiments. Choline *p*-tolyl ether proved to have a short-acting ganglion-blocking effect; hence only single doses of this compound could be used for each comparison, thus lowering the precision of the estimate.

For each set of observations, constituting one comparison of activity, the effect (mm. hypertension) was plotted against log.-dose (in micromolecules), and from the regression lines so obtained, which in all instances appeared to be parallel, the ratio of molar activities was obtained.

Results

The results of all these experiments are collected in Table I for the ethers and analogous compounds, and in Table II for the esters and nicotine. Results for animals with intact suprarenals and those in which these were ligated are given in separate sections. In each section the first column gives the mean doses in μg. molecules, and their standard errors, required to produce a 60 mm. hypertension in the cats (average weight 2.6 kg.) used in the experiments. In the second and third

TABLE I

EQUI-EFFECTIVE DOSES OF CHOLINE ETHERS AND MOLAR ACTIVITIES RELATIVE TO CHOLINE PHENYL ETHER

Compound $R\text{-}O.CH_2CH_2N(CH_3)_3Br$ R =	Suprarenals intact				Suprarenals ligated			
	Dose producing a 60 mm. hypertension		Relative molar activity	Comparisons	Dose producing a 60 mm. hypertension		Relative molar activity	Comparisons
	micromols	μg.			micromols	μg.		
3 : 5-dibromophenyl ..	0.045±0.003	18.4	337	8	0.048±0.005	20.0	268	7
m-bromophenyl	0.041±0.003	13.9	370	6	0.050±0.005	16.9	258	5
m-chlorophenyl	0.069±0.007	20.3	220	5	0.067±0.007	19.7	192	6
phenyl	0.152±0.008	39.5	100	84	0.129±0.009	33.5	100	61
m-tolyl	1.16±0.09	318	13.1	5	0.95±0.09	260	13.5	5
p-chlorophenyl	1.46±0.12	430	10.4	5	1.28±0.08	377	10.1	5
3 : 5-xylyl	2.94±0.41	847	5.2	5	2.48±0.37	714	5.2	6
p-tolyl	38.9±9.1	10,600	0.4	5	27.4±3.9	7,500	0.47	6

β-Phenoxyethyltriethylammonium bromide, γ-phenoxypropyltrimethylammonium bromide, and the phenyl ether of β-methylcholine failed to produce pressor responses. Large doses (2–5 mg.) produced depressor responses and no comparison was therefore possible for these compounds.

TABLE II

EQUI-EFFECTIVE DOSES OF CHOLINE ESTERS AND MOLAR ACTIVITIES RELATIVE TO CHOLINE PHENYL ETHER

Compound	Suprarenals intact				Suprarenals ligated			
	Dose producing a 60 mm. hypertension		Relative molar activity	Comparisons	Dose producing a 60 mm. hypertension		Relative molar activity	Comparisons
	micromols	μg.			micromols	μg.		
Carbachol	2.17±0.35	396	7.0	4	—	—	—	—
Acetylcholine	6.84±2.7	1,240	2.2	4	10.7±2.6	1,940	1.2	4
Propionylcholine	4.47±0.95	1,280	3.4	4	4.94±0.93	1,417	2.6	4
*iso*Butyrylcholine	1.8±0.46	542	8.4	4	1.55±0.26	466	8.3	6
Trimethylacetylcholine ..	0.71±0.15	223	21.4	8	0.62±0.15	195	20.8	5
Benzoylcholine	6.14±0.8	1,495	2.5	5	5.25±0.49	1,280	2.5	5
Phenacetylcholine ..	2.05±0.5	715	7.4	6	1.84±0.27	642	7.0	4
Nicotine acid tartrate ..	0.32±0.08	159	47.5	6	0.22±0.04	109	58.6	4

columns these figures are converted into actual doses in μg. required to produce the same effect and the molar activity ratios compared with choline phenyl ether, which is given an arbitrary value of 100. The figures in the fourth column give the number of comparisons made for each compound ; except that for choline phenyl ether this figure indicates the total number of regression lines drawn.

Since these observations were completed we have had, through the courtesy of Mr. D. P. H. Tudor Williams, the opportunity of examining the compound 4-keto-amyltrimethylammonium iodide. This compound is of especial interest since it represents the replacement of the " ether " oxygen of acetylcholine by a methylene group. Its molar activity, when determined under our standard conditions, is about one quarter that of choline phenyl ether bromide.

Discussion

The results of our experiments, together with those of previous workers, leave little doubt that the polarity of the " ether " oxygen atom of choline derivatives is an important factor in determining the nicotine-like stimulant actions of these compounds, and that reduction of the electron density is associated with increased activity.

This is most clearly seen when the activity of choline *m*-tolyl ether is compared with that of the *m*-chlorophenyl and *m*-bromophenyl ethers of choline. The last two compounds are respectively 16 and 30 times as active as the first and, since they are very nearly isosteric, it is almost certain that charge distribution rather than molecular shape and size is the determining factor. Relationships of the same order also exist between the *p*-tolyl and *p*-chlorophenyl ethers and the 3 : 5-xylyl and 3 : 5-dibromophenyl ethers. The observed activities in the range of choline esters are also substantially in agreement with those which would be predicted.

Certain other factors not considered in the work here described are clearly of importance ; some of these are now being investigated. The most obvious of these is the part played by *ortho*-substitution in choline phenyl ethers. The simple hypothesis would predict activities for choline tolyl ethers *meta*>*ortho* or *para*, whereas Hunt and Renshaw (1936) showed that these were *ortho*>*meta*≫*para*. More recently Loewe, Goodman, and Puttuck (1950) have attributed selective actions on ganglia to certain *ortho*-substituted choline phenyl ethers. Carbamylcholine is also much more active than would be expected, although when N-alkyl substituents are introduced the activity falls (Bender *et al.*, 1943) as would be expected.

Thus, although the hypothesis put forward in this paper seems to have considerable experimental justification it cannot pretend to give a complete picture of this aspect of structure-action relationships in choline derivatives. It is hoped, however, to extend these initial observations so that a more comprehensive knowledge of the subject may be obtained.

Chemical Section

Analyses are by Drs. Weiler and Strauss. Melting and boiling points are uncorrected.

With the exception of the phenyl ether of β-methylcholine, the required compounds were made by boiling an alcoholic solution of the appropriate sodium phenoxide (1 mol.) and the alkylene dibromide (3 mols.). The resulting aryloxyalkyl bromide was then condensed with anhydrous trimethylamine or triethylamine in acetone solution at room temperature, the product crystallizing out in an almost pure condition as it was formed.

TABLE III

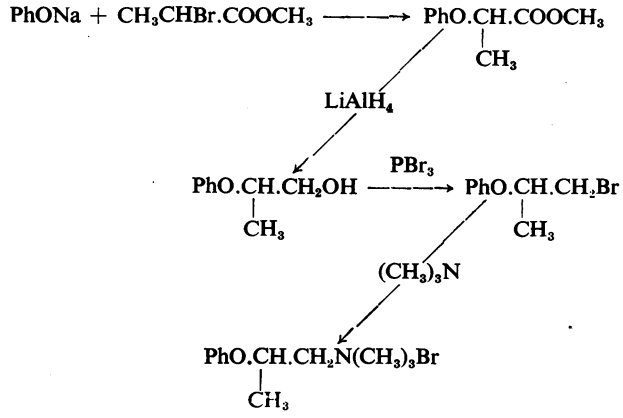
Compound R-O-CH_2CH_2Br R =	Characters	Analysis	Carbon %	Hydrogen %	Halogen %
4-chlorophenyl ..	Oil; b.p. 140°/12 mm. $(n)_D^{20°}$ 1.5685	Found	41.4	3.7	48.3
		Calc. for C_8H_8OBrCl	40.8	3.4	50.0
3-chlorophenyl ..	Oil; b.p. 94°/0.4 mm. $(n)_D^{40°}$ 1.5584	Found	41.1	3.7	49.4
		Calc. for C_8H_8OBrCl	40.8	3.4	50.0
3-bromophenyl ..	Oil; b.p. 145°/14 mm. $(n)_D^{20°}$ 1.5911	Found	34.5	2.9	56.1
		Calc. for $C_8H_8OBr_2$	34.3	2.7	57.1
3 : 5-dibromophenyl	Solid; b.p. 134°/0.9 mm. m.p. 43° (from petroleum ether)	Found	26.9	2.1	67.1
		Calc. for $C_8H_7OBr_3$	26.8	2.0	66.8
3 : 5-xylyl	Oil; b.p. 99°/1.0 mm. $(n)_D^{20°}$ 1.5426	Found	53.5	6.3	34.6
		Calc. for $C_{10}H_{13}OBr$	52.4	5.7	34.9

TABLE IV

Compound R-O-$CH\,CH_2N(CH_3)_3Br$ R =	Characters	Analysis	Carbon %	Hydrogen %	Nitrogen %	Halogen %
4-chlorophenyl ..	Prisms from ethanol; m.p. 172°	Found	45.2	6.1	4.9	38.9
		Calc. for $C_{11}H_{17}ONBrCl$	44.8	5.9	4.8	39.2
3-chlorophenyl ..	Metastable needles from ethanol-acetone; m.p. 131°	Found	44.8	5.7	4.8	39.6
		Calc. for $C_{11}H_{17}ONBrCl$	44.8	5.8	4.8	39.2
3-bromophenyl ..	Prisms from acetone; m.p. 141°	Found	38.9	5.1	4.3	47.5
		Calc. for $C_{11}H_{17}ONBr_2$	40.0	5.0	4.1	47.1
3 : 5-dibromophenyl	Stout metastable needles from ethanol - acetone; m.p. 195.5°	Found	30.9	4.2	3.6	55.6
		Calc. for $C_{11}H_{16}ONBr_3$	31.6	3.9	3.4	57.3
3 : 5-xylyl	Platelets from ethanol; m.p. 218°	Found	54.0	7.6	4.7	28.0
		Calc. for $C_{13}H_{22}ONBr$	54.2	7.7	4.9	27.7

Brief details of compounds made in this way are given in Tables III and IV.

The phenyl ether of β-methylcholine had been previously synthesized by Goldfarb (1941) by a route which did not exclude the possibility of contamination with the extremely active isomeric α-methyl derivative (Hunt and Renshaw, 1936). The following unambiguous synthesis was carried out, the route to 2-phenoxypropan-1-ol appearing to be simpler than that used by Sexton and Britton (1948).



Methyl 2-phenoxypropionate.—Phenol (11.8 g.), dissolved in dry benzene (150 ml.), was heated, under reflux and with stirring, with sodium (2.9 g.) until the latter dissolved, forming a dense suspension of the phenoxide. Methyl 2-bromopropionate (21 g.) was added dropwise with continued heating and stirring during four hours, and the mixture boiled for a further two hours. The sodium bromide was removed by filtration and the filtrate distilled; the required product was a colourless oil, b.p. 120°/14 mm. $(n)_D^{20°}$ 1.5040. Yield 19 g. Found: C, 67.9; H, 7.1. $C_{10}H_{12}O_3$ requires C, 66.7; H, 6.7%.

2-Phenoxypropan-1-ol.—Lithium aluminium hydride (2.5 g.) dissolved in anhydrous ether (250 ml.), was treated dropwise with the above ester (17 g.), dissolved in dry ether (50 ml.), with stirring, so that gentle ebullition was maintained (45 min.). After standing at room temperature overnight the reaction mixture was treated with dilute sulphuric acid to decompose the suspended complex. The ethereal layer yielded the required product as a colourless, somewhat viscous liquid, b.p. 120°/13 mm. $(n)_D^{17°}$ 1.5285. Yield 14 g. Found: C, 71.7; H, 8.2. $C_9H_{12}O_2$ requires C, 71.0; H, 8.0%.

1-Bromo-2-phenoxypropane.—The above alcohol (12.8 g.) was cooled in ice and treated with phosphorus tribromide (7.6 g.); the mixture was then allowed to come to room temperature. After standing for 24 hr. it was heated to 100°, shaken with water and extracted with ether. Distillation gave the required product, b.p. 108°/12 mm. $(n)_D^{20°}$ 1.5424. Yield 12 g. Found: C, 50.6; H, 5.6; Br, 37.5. $C_9H_{11}OBr$ requires C, 50.3; H, 5.2; Br, 37.2%.

Phenyl ether of β-methylcholine bromide.—1-Bromo-2-phenoxypropane (11 g.) and trimethylamine (5 g.) in acetone (5 ml.) gave (15 days) 10 g. of required product. Recrystallization from alcohol, acetone, ethyl acetate, and ethyl cellosolve gave products of indefinite melting point, probably owing to inclusion of solvent. Removal of solvent by heating *in vacuo* gave a product melting sharply at 166°. Found: C, 52.0; H, 7.3; N, 4.9; Br, 28.1. $C_{12}H_{20}ONBr$ requires C, 52.5; H, 7.3; N, 5.1; Br, 29.1%.

β-Phenoxyethyltriethylammonium bromide, γ-phenoxytrimethylammonium bromide, and the other choline phenyl ethers were made by methods already described in the literature. The choline esters used were made by esterifying ethylene iodohydrin with the appropriate acid chloride, and condensing the β-iodoethyl ester with anhydrous trimethylamine in acetone solution.

SUMMARY

1. A hypothesis is proposed relating certain aspects of chemical structure to nicotine-like stimulant actions in choline derivatives. This states that increased activity is associated with a reduction of the electron density of the " ether " oxygen atom of choline ethers and esters.

2. The hypothesis is supported by much evidence from the literature, which is briefly reviewed.

3. Further supporting evidence is afforded by a quantitative pharmacological study of twenty-one compounds, several of which are new. Three of these (the *m*-chlorophenyl, *m*-bromophenyl and 3 : 5-dibromophenyl ethers of choline) have two or three times the molar activity of choline phenyl ether, the most powerful nicotine-like stimulant drug hitherto described which, in turn, has about twice the molar activity of nicotine.

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