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Two Cyclic Analogs of Acetylcholine*

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Cyclic compounds which imitate two spatial configurations of acetylcholine have been studied as to their muscarinic activities and hydrolyzability by acetylcholinesterase. The results lend support to the hypothesis that flexibility *per se* is a factor to be considered in the collation of chemical structure with pharmacologic activity.

MATHEMATICAL and experimental studies concerning diverse congeners of acetylcholine indicate that the collation of chemical structure with muscarinic or neuromuscular blocking activity is more reliable when drawn in terms of a statistical distribution of the steric factors involved than when single measurements serve as the basis for structural comparison (1, 2). Indeed, among the chainlike congeners of acetylcholine and decamethonium, flexibility *per se* (i.e., a wide distribution of molecular length) appears to be an important factor for high potency. Whether this latter arises out of a corresponding variability in the spacing of the primary receptor sites is, of course, unknown.

The present report emphasizes the above conception of flexibility as a factor pertinent to high muscarinic activity through the consideration of the pharmacologic activities of two cyclic analogs of acetylcholine (Fig. 1). These materials, I and II, are of special interest in so far as they are relatively rigid molecules analogous to the extreme configurations, III and IV, assumable by acetylcholine. The determination of the muscarinic potency of the analogs I and II, relative to acetylcholine, together with an assessment of their susceptibility as substrates of bovine erythrocyte cholinesterase were carried out.

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EXPERIMENTAL

Materials.—The cyclic analog I, N,N-dimethyl-6-morpholonium iodide, was synthesized according to the scheme in Fig. 2. Five-tenths mole monochloroacetic acid and 1.0 *M* β-methylaminoethanol were mixed in a beaker cooled in an ice-water mixture. After the rapid exothermic reaction was over, the resulting thick syrup was allowed to stand overnight at room temperature. Then, following solution in an equal volume of water, 1.0 *M* sodium hydroxide (as a 40% solution) was added. The resulting mixture, after cooling, was extracted six times using for each extraction a volume of ether equal to the volume of the mixture. This treatment removes the excess 0.5 *M* of β-methylaminoethanol. The ether extract was discarded and the residue evaporated nearly to dryness on the water bath, cooled and finally treated with a sixfold volume of acetic anhydride. Following the initial reaction with acetic anhydride, during which some heat is evolved, the whole mixture was boiled for a few minutes and allowed to stand overnight, during which time a quantity of fine crystals was deposited (NaCl and NaAc). The crystals were removed by filtration and the filtrate subjected to distillation at atmospheric pressure (about 740 mm. of Hg). After a forerun of acetic anhydride and acetic acid a somewhat viscous oil was obtained over the temperature range 225–240°. This oil was redistilled, the portion boiling between 235–240° being collected. The oil, which is colorless when pure, has a slight ammoniacal odor. Quaternization of the oil was carried out by solution in an equal volume of ether followed by the addition of

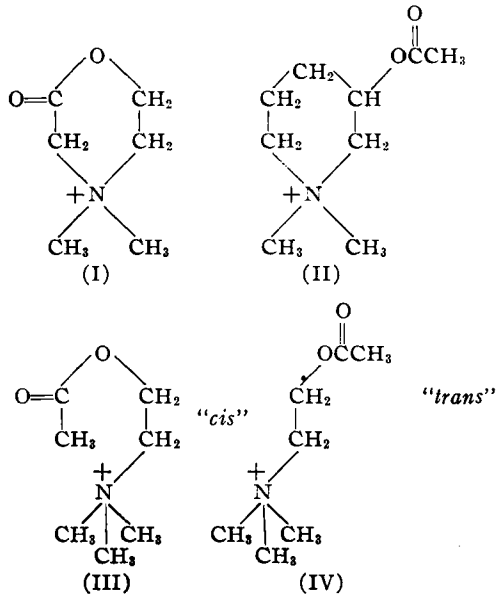

Fig. 1.—Comparison of the cyclic analogs with corresponding configurations of acetylcholine.

excess methyl iodide, the mixture being allowed to stand at least twenty-four hours. The white crystalline solid obtained was filtered off and washed repeatedly with peroxide-free dry ether and, finally, dried *in vacuo*. This substance obtained in an overall yield of about 30%, is freely soluble in water, and has a m. p. 239–240° (with decompn.).

Anal.—Calcd. for $C_6H_{12}O_2NI$: I, 49.21. Found: I, 49.4.

Determination of the acid (lactone) equivalent, by digestion of a 15-mg. sample in 10 cc. of 0.01 *N* sodium hydroxide followed by back titration with 0.01 *N* hydrochloric acid using phenolphthalein as an indicator, checked within 2% of the theoretical value.

A sample of analog II (Fig. 1), N,N-dimethyl-β-acetoxypiperidinium bromide, was donated by Lakeside Laboratories, Inc.

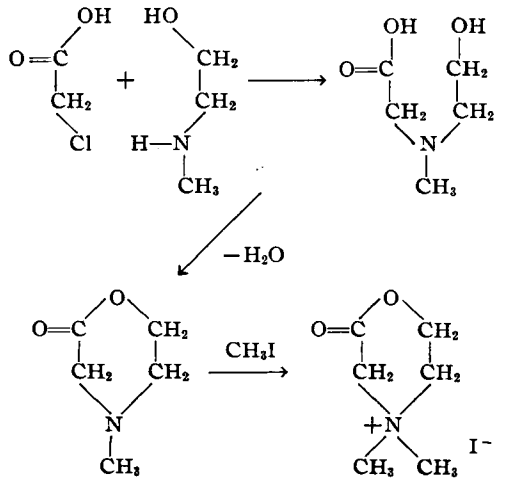

Fig. 2.—Scheme for the synthesis of cyclic analog I.

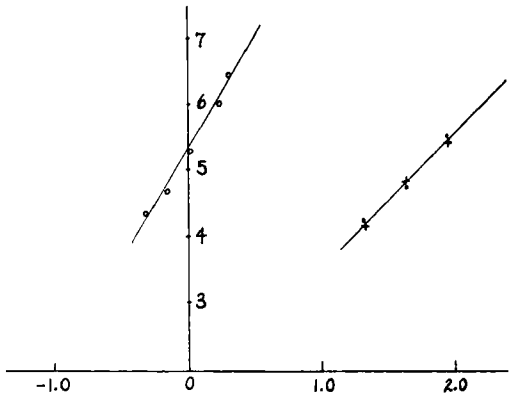

Fig. 3.—Dose-depressor response relationships on the dog arterial blood pressure: abscissa in $\log_{10}$ (dose μg./Kg.), ordinate in probits. +, ●, ○ denote, respectively, analogs I, II, and acetylcholine.

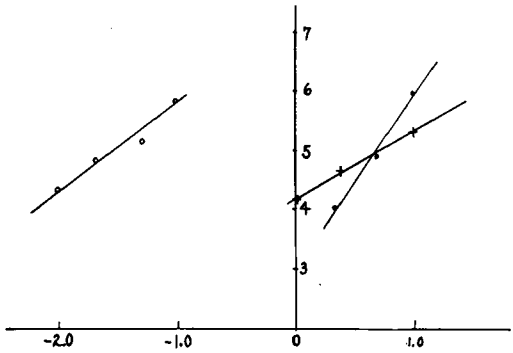

Fig. 4.—Dose-depressor response relationships (as in Fig. 3) following neostigmine bromide.

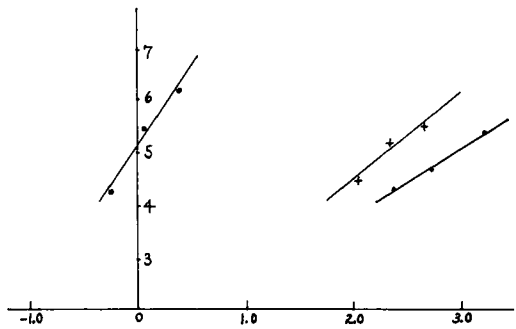

Fig. 5.—Dose-contraction response relationships using the isolated gut of the guinea pig. Abscissa in $\log_{10}$ (concentration in μg. %), ordinate in probits. Labeling as in Figs. 3 and 4.

Methods.—Using acetylcholine chloride as a standard, the products in question were evaluated in regard to their muscarinic depressor and gut stimulant activities following methods which have already been described in detail (3). Conventional manometric methods were used in the determination of their susceptibility as substrates for bovine

TABLE I.—RELATIVE MOLAR DEPRESSOR POTENCIES

	ED_{50} (μM/Kg.)		Activity Ratio[b]		
	Before Neostigmine[a]	After Neostigmine	Before Neostigmine	After Neostigmine	Potentiation Ratio[c]
Acetylcholine chloride	0.0035	0.000115	1	1	30.4
I	0.18	0.017	0.019	0.0068	10.1
II	0.23	0.018	0.015	0.0064	12.8

[a] The potentiating dose of neostigmine was 30 μg./Kg.

[b] Activity ratio = $\frac{ED_{50}\text{ acetylcholine}}{ED_{50}\text{ drug}}$.

[c] Potentiation ratio = $\frac{ED_{50}\text{ before neostigmine}}{ED_{50}\text{ after neostigmine}}$.

erythrocyte cholinesterase with acetyl-β-methylcholine as a standard (4).

All dose-response relationships were calculated and plotted in terms of probit-response *versus* $\log_{10}$ (dose in μg./Kg.). The curves so obtained were, in general, linear though often not parallel.

RESULTS AND DISCUSSION

Figures 3 and 4 represent the muscarinic depressor activity before and after neostigmine. Figure 5 represents the gut stimulant activity using the isolated ileum of the guinea pig and Fig. 6 the enzymic and spontaneous hydrolyzability. The depressor and gut stimulant responses caused by the analogs I and II were always effectively blocked by doses of atropine sulfate sufficient to inhibit the muscarinic responses to acetylcholine.

Table I exhibits the depressor effects of the analogs I and II relative to acetylcholine when the ED_{50} for each is calculated from the primary graphs on a molar basis. The drugs I and II were strikingly similar in depressor potency before and after the administration of neostigmine. Moreover, the degree of potentiation effected by neostigmine for both cyclic agents was of the same order of magnitude though neither agent was potentiated to as high a degree as acetylcholine.

As gut stimulants, analogs I and II, were also much less potent than acetylcholine though, in this test, analog I was somewhat more active than II (Fig. 5). Their susceptibilities to hydrolysis by cholinesterase are again similar (Fig. 6), but analog I was hydrolyzed very rapidly in a nonenzymatic fashion at the *p*H of the system (7.4) whereas analog II was relatively stable.

Analogs I and II represent, respectively, relatively rigid configurations assumable by the flexible molecule of acetylcholine. If analog I can be thought of as a material in which a "*cis*" type of configuration has been enforced through cyclization, analog II would represent a corresponding extended or "*trans*" form.

The *enforcement* of neither configuration appears to have significantly greater advantage over the other in enhancing muscarinic activity or susceptibility to cholinesterase hydrolyzability. Both situations, representing as they do a decrease in flexibility, confirm the results obtained previously (1) through the investigation of open chain analogs of II, that the flexibility of acetylcholine, as such, is an important attribute concerning the relationship between its structure and action. Whatever importance, therefore, is to be assigned to the nitrogen-ester group, intramolecular distance is best considered as a statistical distribution which may match an equivalent variability found in the receptors with which acetylcholine interacts.

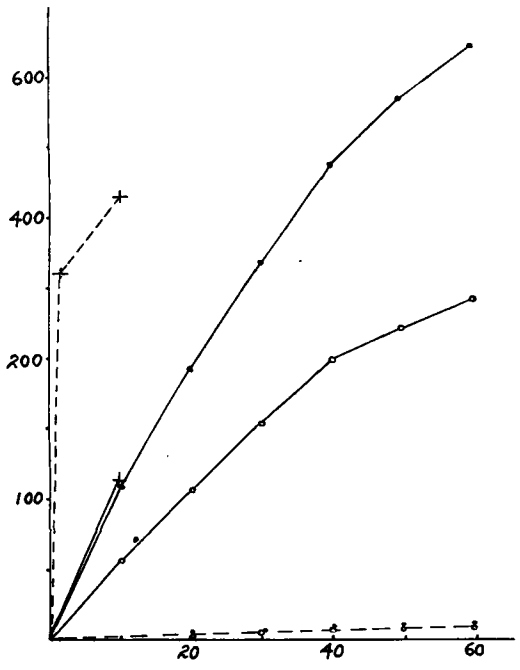

Fig. 6.—Enzymic (solid lines) and spontaneous hydrolysis (dash lines) abscissa in minutes, ordinate in total μL. of CO_2 evolved. +, ●, and ○ represent, respectively, analogs I, II, and acetyl-β-methylcholine.

SUMMARY

Two cyclic analogs of acetylcholine have been evaluated in regard to their muscarinic depressor and gut stimulant effects. The data lend support to the conjecture that flexibility of the acetylcholine molecule is an important attribute to be considered in collating structure with pharmacologic activity.

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