Cholinergic Receptor I. Cholinomimetic Activities of Some Analogs of *cis*-2-Methyl-4-dimethylaminomethyl-1,3-dioxolane Methiodide

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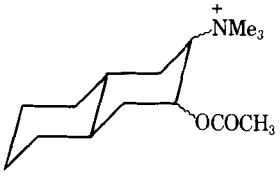
The cholinomimetic activities of acetylcholine, *cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide, *N*-methyl-6,8-dioxa-3-azabicyclo[3.2.1]octane methiodide, and *N*-methyl-7,9-dioxa-3-azabicyclo[4.2.1]nonane methiodide have been compared and an interpretation of their relative activities in terms of a conformation of receptor-bound cholinomimetic molecules has been put forward. *N*-Methyl-7,9-dioxa-3-azabicyclo[4.2.1]nonane methiodide has been shown to have some acetylcholine releasing activity.

A FUNDAMENTAL assumption in the analysis of structure–activity relationships is that mutual complementarity exists between the drug and receptor. With this assumption, analysis of structure–activity relationships should yield information relevant to an understanding of the binding sites of the receptor. However, with flexible molecules there is no reason to assume that the conformation of lowest free energy in the solution or crystalline state is necessarily that which is involved in binding at the receptor surface. In the absence of evidence concerning the conformation of receptor-bound molecules, analysis of structure–activity relationships in terms of the relative geometry of binding sites becomes equivocal.

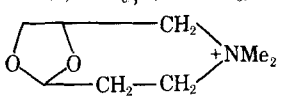
A partial solution to this problem may be obtained through the use of conformationally rigid analogs of active molecules where the distances between potentially important binding groups are fixed. A number of workers have used this approach in attempts to define more precisely the structure of the cholinergic receptor (1, 2). Most recently, Smissman, LaPidus, and their co-workers (3) have studied the four isomeric 2-acetoxy-3-trimethylammonium-*trans*-decalins (I*a*,*b*,*c*,*d*), which contain the basic acetylcholine structure, and found them to have negligible muscarinic activity. However, the observed inactivity of these compounds may well be due, not to a failure to reproduce the geometry of receptor-bound acetylcholine, but to the incorporation of additional potential binding groups—specifically the large hydrocarbon skeleton—a feature which has been repeatedly demonstrated to depress cholinomimetic activity and convert agonists to antagonists (4, 5).

This latter point is considered to be of substantial importance. Accordingly, in this approach to the design of semirigid and rigid analogs of acetylcholine the authors have endeavored to meet the requirements of minimum conformational flexibility without the incorporation of additional potential binding groups. In this paper are reported the synthesis

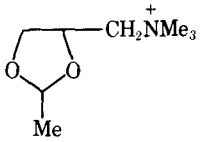


II

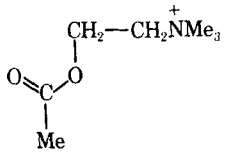
I*a* (*a*, NMe_3; *a*, $OCOCH_3$)
b (*a*, NMe_3; *e*, $OCOCH_3$)
c (*e*, NMe_3; *e*, $OCOCH_3$)
d (*e*, NMe_3; *a*, $OCOCH_3$)



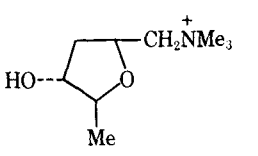
III



IV



V



VI

and cholinomimetic activity of two bicyclic compounds (II and III) which are related to *cis*-2-methyl - 4 - dimethylaminomethyl - 1,3 - dioxolane methiodide (IV) whose high muscarinic activity was first reported by Triggle and Belleau (6). *N*-Methyl - 6,8 - dioxa - 3 - azabicyclo[3.2.1]octane methiodide (II) was first reported by Fourneau and Chantalou (7) but adequate comparative pharmacological data were not presented. The close structural resemblance of IV to both acetylcholine (V) and muscarine (VI) is of considerable interest and has been noted earlier by several workers (6, 8–10).

EXPERIMENTAL

Chemistry

Melting points were determined on a Thomas-Kofler hot stage and are corrected. GLC analyses were carried out with a Varian Aerograph (model A-90-P). Analyses are by Dr. A. E. Bernhardt, Mulheim, Ruhr, West Germany.

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cis - 2 - Methyl - 4 - dimethylaminomethyl - 1,3-dioxolane Methiodide (IV)—This was prepared by the method of Triggle and Belleau (6) and had m.p. 144° [lit. (6) m.p. 143–144°].

N - Methyl - 6, 8 - dioxa - 3 - azabicyclo[3.2.1]-octane Methiodide (II)—This was prepared by the method of Fourneau and Chantalou (7). *cis,-trans* - 2 - Bromomethyl - 4 - chloromethyl - 1,3-dioxolane was prepared from bromoacetaldehyde dimethyl acetal and glycerol α-monochlorhydrin (7) and was shown by GLC to consist of a 3:2 mixture of the *cis,trans* isomers. *cis,trans*-2-Bromomethyl - 4 - chloromethyl - 1, 3 - dioxolane (10.2 Gm., 0.05 mole) and methylamine (7.8 Gm., 0.25 mole) in benzene (100 ml.) were heated at 100° for 12 hr. On cooling, the mixture was filtered from methylamine hydrohalides and fractionally distilled to give *N*-methyl-6,8-dioxa-3-azabicyclo[3.2.1]octane (3.5 Gm., 87% based on *cis*-content of *cis,-trans* - 2 - bromomethyl - 4 - chloromethyl - 1,3-dioxolane) with b.p. 75°/30 mm.

Anal.—Calcd. for $C_6H_{11}NO_2$: C, 55.78; H, 8.57; N, 10.84. Found: C, 55.81; H, 8.72; N, 10.76.

N-Methyl-6,8-dioxa-3-azabicyclo[3.2.1]octane was quaternized by treatment with excess methyl iodide in ether to give II in 95% yield [MeOH–EtOH] with m.p. 252° [lit. (7) m.p. 250° dec.].

Anal.—Calcd. for $C_7H_{14}INO_2$: C, 31.12; H, 4.85; I, 46.97; N, 5.18. Found: C, 30.81; H, 5.15; I, 46.79; N, 5.00.

N - Methyl - 7,9 - dioxa - 3 - azabicyclo[4.2.1]-nonane Methiodide (III)—This was prepared by a method similar to that described for II. *cis,trans*-2-Chloroethyl-4-chloromethyl-1,3-dioxolane was prepared from β-chloro-propionaldehyde trimer (55.2 Gm., 0.2 mole, from Shell Development Corp.) and glycerol α-monochlorhydrin (22.2 Gm., 0.2 mole) by refluxing in benzene (300 ml.) in the presence of *p*-toluenesulfonic acid (0.2 Gm.) with continuous removal of water. Fractional distillation gave the product in 85% yield with b.p. 80–85°/0.2 mm., which was shown by GLC to consist of a 3:2 mixture of *cis,trans* isomers.

Anal.—Calcd. for $C_6H_{10}Cl_2O_2$: C, 38.93; H, 5.44; Cl, 38.31. Found: C, 38.81; H, 5.41; Cl, 38.21.

cis,trans - 2 - Chloroethyl - 4 - chloromethyl - 1,3-dioxolane (9.2 Gm., 0.05 mole) and methylamine (7.8 Gm., 0.25 mole) in benzene (100 ml.) were heated at 100° for 16 hr. On cooling the mixture was filtered from methylamine hydrochloride and fractionally distilled to give *N*-methyl-7,9-dioxa-3-azabicyclo[4.2.1]nonane (3.8 Gm., 91% based on presumed *cis*-content of *cis,trans*-2-chloroethyl-4-chloromethyl-1,3-dioxolane) with b.p. 85–87°/25 mm.

Anal.—Calcd. for $C_7H_{13}NO_2$; C, 58.73; H, 9.15; N, 9.78. Found: C, 58.51; H, 9.01; N, 9.71.

The *N* - methyl - 7,9 - dioxa - 3 - azabicyclo[4.2.1]-nonane was quaternized with methyl iodide to give III in 91% yield with m.p. 244–246° [MeOH–EtOH].

Anal.—Calcd. for $C_8H_{16}INO_2$: C, 33.71; H, 5.65; I, 44.51; N, 4.91. Found: C, 33.78; H, 5.64; I, 44.38; N, 4.74.

PHARMACOLOGY

Muscarinic activities of the compounds were determined using the rat jejunum. Male rats (250 Gm.) were decapitated, bled, and a length of jejunum removed and washed through with Tyrode's solution. Short lengths (3 cm.) were then suspended in oxygenated Tyrode solution in 20-ml. muscle baths with the temperature maintained at 37°. The tissues were allowed to relax for 1 hr. and cumulative dose-response curves for the compounds II, III, and IV were obtained. Dose-response curves for acetylcholine were determined simultaneously using jejunum segments from the same animal. Compounds were added in saline to give the expressed final bath concentration and isometric contractions were recorded on a Grass polygraph (model 5.D).

Nicotinic activities were determined using the frog rectus abdominus preparation. The isolated muscle was suspended in oxygenated Ringer's solution at room temperature in 20-ml. muscle baths and sensitized with eserine ($3 \times 10^{-5}M$, final bath concentration) for 20 min. Dose-response curves were determined and isometric contractions recorded with a Grass polygraph.

Acetylcholine release from the rat jejunum was determined essentially according to the method of Schaumann (11). (See also *Reference 12.*) Jejunum from three freshly decapitated rats were washed through with Tyrode's solution and cut into segments of 1.5 cm. length. To one portion (5 Gm.) suspended in oxygenated eserinized ($3 \times 10^{-5}M$) Tyrode's maintained at 35° (20 ml.) was added III to give a final concentration of 10^{-4} Gm./ml. and at 15, 30, and 45 min. additional equal amounts of III were added to give a final bath concentration of 4×10^{-4} Gm./ml. Another portion (5 Gm.) of jejunum served as a control and was treated identically save that Tyrode's was added instead of III. The incubation mixtures were then centrifuged and the supernatant fluid was assayed for acetylcholine-like activity on the rectus abdominus preparation described above.

RESULTS

Muscarinic and Nicotinic Activities—The pharmacological activities are given in Table I. The results confirm the observation of Triggle and Belleau (6) who found *cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide (IV) to be as potent as acetylcholine in the guinea pig ileum preparation: IV is found to be only slightly less potent than

Table I—Muscarinic and Nicotinic Activities

Compd.	Rat Jejunum, ED_{50} Gm./20 ml.	Relative *M* Potency	Frog Rectus, ED_{50} Gm./20 ml.	Relative *M* Potency
Acetylcholine	$2.2 \pm 1.8 \times 10^{-7}$	1.0	$3 \pm 0.5 \times 10^{-6}$	1.0
II	Inactive at 10^{-3}	...	Produced 8% response at 10^{-4}	...
III	$2.7 \pm 4.7 \times 10^{-5}$	0.012[a]	Inactive at 5×10^{-4}	...
IV	$4.0 \pm 3.2 \times 10^{-7}$	0.75	$9 \pm 1.0 \times 10^{-5}$	0.049

[a] Produced only 75% of the maximum response (ACh = 100).

acetylcholine on the rat jejunum preparation and to have only one-twentieth of the nicotinic activity of acetylcholine on the frog rectus preparation. The bicyclic analogs, II and III, of IV were found to have negligible activities at both muscarinic and nicotinic receptors.

Acetylcholine Releasing Activities—Reproducible dose-response curves could not be obtained with III on the rat jejunum preparation. The maximum response elicited decreased with repetition of the dose-response curve until after 4–5 such determinations the response remained steady at 45–55% of the original maximum response. This behavior was in marked contrast to acetylcholine and IV which gave 6–8 highly reproducible dose-response curves over a 60–90 min. period. It was found that III was able to release acetylcholine from rat jejunum. As determined by bioassay on the frog rectus abdominus preparation, III (4×10^{-4} Gm./ml.) liberated 4.2 ± 2.4 mcg./Gm. tissue/hr. compared to a spontaneous release of 1.5 ± 1.08 mcg./Gm. tissue/hr. (P value, 0.05–0.02).

DISCUSSION

The finding that the bicyclic analogs II and III of IV have negligible activities and that, in fact, II is apparently devoid of muscarinic activity is of some interest. This finding clearly suggests that IV, and by analogy acetylcholine, acetyl-β-methylcholine, and muscarine, do not adopt, when bound at the muscarinic receptor, any of the limiting conformations represented by II and III. Whether this is related to the inactivity of II and III remains to be established through the synthesis of further bicyclic analogs of this type (14).

The complete inactivity of II at the muscarinic receptor also relates to the proposed modes of binding of muscarine and muscarone at cholinergic receptors. Waser (9) has suggested that, in order to account for the relative loss and inversion of optical specificity of muscarone relative to muscarine, D-muscarone is bound at the receptors in a conformation in which the trimethylammonium group is symmetrically disposed over the ether and carbonyl oxygen functions; the carbonyl group is assumed to be able to bind equivalently to the receptor site which normally binds the ether oxygen of L-muscarine. According to Waser's argument, II should be extremely active, since in one of its limiting conformations the quaternary ammonium group is centered symmetrically over the two electronically equivalent ether oxygens (the latter feature is not really true in Waser's proposed conformation for D-muscarone). Its complete inactivity reinforces the earlier arguments of Belleau and Puranen (15), based on the relative stereospecificity of L- and D-*cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide, concerning the difficulties with Waser's interpretation of the binding of D-muscarone at the muscarinic receptor and provides further support for their suggestion that the carbonyl group in muscarone binds to an accessory nucleophilic site at the receptor thus introducing a binding characteristic that is not available for muscarine.

The remaining point of interest in connection with these compounds concerns the acetylcholine releasing activity of III. This activity, while relatively feeble, may be related to the steady decrease in maximum response obtained with III on repeated administration and raises again the question that certain cholinomimetic compounds may act, at least in part, by releasing acetylcholine from peripheral storage sites (16–18).

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Keyphrases

Cholinergic receptor
cis-2-Methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide analogs—synthesis
Cholinomimetic activity
Muscarinic activity
Nicotinic activity
GLC—analysis