Relation of Structure of Synthetic Muscarines and Muscarones to Their Pharmacological Action.* (24216)

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Muscarine was first extracted from *Amanita muscaria* by Schmiedeberg and Koppe (1). The chemical structure proposed by Kögl *et al.*(2) was shown to be in error when Eugster and Waser(3) and Eugster(4) isolated pure, crystalline muscarine, determined the empirical formula, $C_9H_{20}O_2NCl$, and obtained evidence for presence of a tetrahydrofurane ring. Kögl *et al.*(5) established by X-ray crystallography the structure of muscarine as a stereoisomeric form of 2-methyl-5-dimethylamino-methyl - tetrahydrofurane - 3-ol methochloride. As muscarine belongs to a group of tetrahydrofurane compounds with 3 asymmetric carbon atoms, 8 stereoisomeric forms are possible. The first syntheses of muscarine resulted in mixtures of stereoisomers(6). A stereospecific synthesis of L-(+) muscarine was reported by Hardegger and Lohse(7). More recently, the synthesis of all racemic stereoisomers of muscarine has been accomplished(8). The present study aims to correlate muscarinic action to stereostructure by comparing pharmacologically (a) natural and synthetic L-(+) muscarine (b) L-(+) muscarine with other muscarine isomers (c) these muscarines with some closely related muscarone compounds.

Materials. The following compounds were studied: L-(+) muscarine chloride, natural, Fig. 1.A(3); L-(+) muscarine chloride, synthetic(9); D-(−) muscarine chloride(9); dl-muscarine chloride(10); and dl-muscarine iodide(10); dl-epi-muscarine iodide, Fig. 1,B (10); dl-allo-muscarine iodide, Fig. 1,C(11); dl-epiallo-muscarine iodide, Fig. 1,D(8); (+) - muscarone iodide ($[\alpha]_D$ + 11.2°, water) and (−) muscarone iodide ($[\alpha]_D$ - 11.5°, water), Fig. 1,E, prepared through resolution of dl-normuscarone(10) with the aid of di-p-toluyl-tartaric acids and quaternization of the (+) and (−) normuscarones (Eugster *et al.*, unpublished); dl-muscarone iodide(10); dl-allo-muscarone iodide, Fig. 1,F(11); dl-4,5-dehydro muscarone chloride, Fig. 1,G(10).

Methods. Pelvic nerve-bladder preparation of the dog. The bladder of female dogs under pentobarbital anesthesia was cannulated and connected with water manometer. The pelvic nerve was electrically stimulated with condenser discharge pulses (frequency 40-200/sec. duration of pulses 1 msec., voltage 1-10 V.) every 10 sec. for 1/5 second duration to avoid spontaneous movements of bladder. The compounds were administered through the cannulated left iliac artery into the abdominal aorta employing of each compound 2 doses differing by a ratio of 1:2. Each compound was tested in 3 to 7 experiments. Mean equiactive dose ratios relative to muscarine chloride, and their standard deviations were calculated; doses were expressed as number of molecules of a compound rather than on a weight basis. The effective dose range of muscarine was between 0.025-0.4 μg/kg. *Isolated rabbit ileum.* Segments of rabbit ileum were suspended in tissue bath according to the method of Magnus. 2 x 2 point assays were performed in 4 to 7 experiments with each compound. Mean equiactive dose ratios relative to muscarine and their standard deviations were calculated. Muscarine was effective in concentrations of 0.005 to 0.02 μg/ml. *Miosis in mice.* The method of Shingh Grewal(12) using albino mice was modified in that mecamylamine (5 mg/kg, intraperitoneally) instead of atropine was given to dilate the pupil. The muscarines were given intraperitoneally, simultaneously with mecamylamine, in 3 logarithmically spaced doses to groups of 6 mice each. The pupil size was determined after 15 and 30 minutes. The potencies of compounds were expressed by equiactive dose ratios and their

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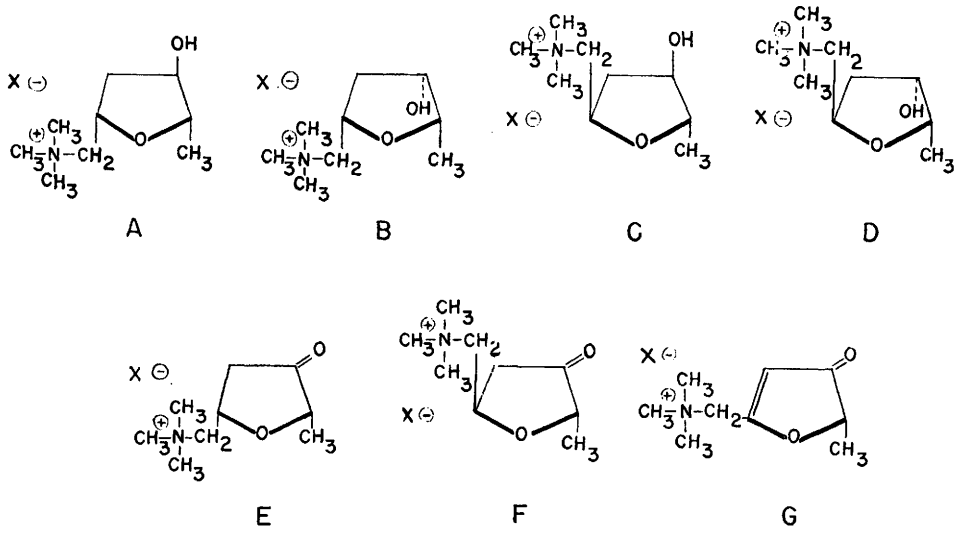

FIG. 1. Steric structures of muscarine and muscarone homologues.

fiducial limits relative to muscarine chloride. *Frog rectus abdominis muscle.* The rectus muscle of *Rana pipiens* was suspended in frog Ringer solution at room temperature. Contractures were recorded isometrically *via* a strain gage and a polygraph. Since muscarine-like compounds have a slower onset of action than acetylcholine, each drug was in contact with the tissue for 4 to 5 minutes. The compounds were given at 2 or 3 dose levels, and the approximative equiactive doses relative to acetylcholine chloride were calculated on the basis of 2 to 4 experiments.

Results. Table I presents the data obtained on postganglionic parasympathetic action of muscarine compounds on 3 types of smooth muscle. Relative potency of the compounds determined in dog bladder preparations closely agrees with that obtained in tests on the isolated ileum and on the pupil. In all 3 tests synthetic muscarine was equal in potency to natural muscarine.

D-(−) muscarine has been found at least 200-800 times less potent than the L-(+) form. It has been reported to have less than 5% of the potency of the L-(+) form when tested on the cat's blood pressure(9), and to be inactive on the isolated frog heart(13). Racemic muscarine was 2.5 times less active than the L-(+) form indicating also that the D-(−) form is inactive.

The allo, epi, and epiallo isomers are several hundred times less potent than racemic muscarine; dl-epimuscarine being the least potent isomer.

Table I also presents data on the action of close congeners of muscarine in which a keto group replaces the hydroxyl group. These muscarones are more active than the corresponding muscarines. Racemic muscarone iodide is about 8 times more potent than dl-muscarine iodide. The optically active (−) form is more active, the (+) form only slightly less active than the racemate. In contrast to the findings on dl-allo-muscarine, dl-allo-muscarone is only slightly less potent than dl-muscarone. The unsaturated 4,5-dehydro muscarone is about twice as potent as dl-muscarine.

The results of tests on the frog rectus muscle (Table I) show that the nicotinic potency of the alkaloids of the muscarine group is negligible; it is at least a few hundred times less than that of acetylcholine. In contrast, the compounds of the muscarone group cause appreciable neuromuscular stimulation. They surpass acetylcholine in potency.

Discussion. The experiments demonstrate a definite stereospecificity of the action of the muscarines on postganglionic parasympathetic effector sites: epi, allo, epiallo muscarines possess only a negligible fraction of the

TABLE I. Comparative Potency of Muscarine and Muscarone Homologues.

Compounds	No. of molecules equivalent to 1 molecule of natural muscarine chloride									No. of molecules equivalent to 1 molecule of acetylcholine
	Dog bladder			Isolated rabbit ileum			Mouse miosis			Frog rectus abdominis muscle
L-(+) muscarine chloride, natural	1.0			1.0			1.0			>400 (3)†
Idem, synthetic	.9	.042*	(6)†	.95	.055*	(7)†	.86 (.58–1.21)‡			>200 (2)
D-(−) muscarine chloride	~800			~400			>200			
dl-muscarine chloride	2.55	.43	(4)	2.4	.24	(4)	2.3 (1.5 –4.0)			>400 (2)
dl-muscarine iodide	2.77	.34	(6)	3.1	.24	(6)	1.6 (.8 –2.5)			>600 (2)
dl-epi-muscarine iodide	1700		(3)	>700		(3)	>600			" (2)
dl-allo-muscarine iodide	500		(3)	460	60	(4)	>600			" (2)
dl-epiallo-muscarine iodide	365	111	(4)	660	145	(4)	>140			>280 (2)
+ Muscarone iodide	.52	.59	(4)	.45	.07	(6)	.32 (.15– .58)			.5 (2)
− Muscarone iodide	.17	.019	(4)	.19	.019	(6)	.19 (.12– .28)			.3 (2)
dl-muscarone iodide	.35	.009	(4)	.4	.05	(7)	.2 (.11– .3)			.5 (3)
dl-allo-muscarone iodide	.77	.05	(4)	.84	.24	(6)	.97 (.7 –1.2)			.3 (2)
dl-4,5-dehydro-muscarone chloride	1.62	.33	(6)	1.5	.25	(6)	3.0 (2.3 –3.9)			.5 (4)

* ± stand. dev. of mean equiactive dose ratio. † No. in parentheses = No. of experiments.
‡ Fiducial limits.

potency of the dl-muscarine. Furthermore, the enantiomers of muscarine differ greatly in their potency; the potency seems to reside almost exclusively in the L-(+) form. All stereoisomers of muscarine are devoid of significant action on the skeletal muscle.

On the contrary, a stereospecificity of the pharmacological action cannot be demonstrated with the enantiomers and the allo isomer of dl-muscarone; these muscarones did not differ greatly among each other in their action on smooth muscle. There were, however, potent stimulators of skeletal muscle, at least equal to acetylcholine in causing contracture of the frog's rectus muscle. Thus, dl-muscarone was 8 times were potent than dl-muscarine on smooth muscle; on skeletal muscle it was more potent than acetylcholine.

Substitution of the hydroxyl group of muscarine by a keto-oxygen also involves a reduction from 3 asymmetric to 2 asymmetric carbon atoms. Thus, it cannot be stated that either one of these changes is responsible for the difference in stereospecificity and in nicotinic action between muscarines and muscarones. It is tempting to speculate that the pronounced nicotinic potency of the muscarones results from introduction of the keto group *per se;* the muscarones with the keto oxygen-ether group bear a greater structural resemblance to acetylcholine than does muscarine with the hydroxyl-ether group. It is interesting to note that 4,5-dehydromuscarone possesses less muscarinic but somewhat greater nicotinic potency than dl-muscarone.

Summary. 1. Comparison of the potency of muscarine and synthetic congeners on smooth muscle (gut, bladder, iris) and on skeletal muscle demonstrated equal potency of synthetic and natural L-(+) muscarine. 2. The potency of muscarine is stereospecific;

D-(−) muscarine and all racemic stereoisomers (epi, allo, epiallo) possess only a fraction of the potency of dl-muscarine. 3. Keto analogues of muscarine (muscarones) are more potent than muscarine in stimulating smooth muscle; in addition, they possess, on frog skeletal muscle, remarkable nicotinic activity of which the muscarines are almost devoid. 4. The potency of the muscarones is not stereospecific. Both + and − muscarones are more potent than L-(+) muscarine, and dl-allo muscarone is only slightly less potent than dl-muscarone.

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Alterations in Serum Proteins of Hibernating Hamsters.* (24217)

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In view of the manifold metabolic adjustments which accompany natural hibernation (1,2,7) it is not unreasonable for one to expect an alteration in the partitions of serum proteins during this state. That certain such changes may occur in at least the hibernating hedgehog (*Erinaceus europeueus*) was shown by Biörck *et al.*(3) whose data also indicated that there was no change in total serum protein level during hibernation in spite of a marked increase in hematocrit. The primary aim of our work was to determine what changes take place in serum proteins upon assumption of hibernation by the golden hamster (*Mesocricetus auratus*). We also have attempted to follow these changes in small series of cold exposed hamsters prior to onset of hibernal sleep and following spontaneous arousal therefrom.

Materials and methods. Other than control hamsters, maintained at room temperature, all animals were kept in a temperature controlled room at 5 ± 0.5°C. Experimental animals were divided into 3 groups: *Group 1* was exposed to the cold for various intervals of time, but had not hibernated; *Group 2* had hibernated continuously for 2 days; *Group 3* had been awake in the cold for one and 2 days after *spontaneous* arousal from hibernation. All animals were killed by cervical fracture. Blood for serum electrophoresis and hematocrit determinations was obtained directly from right ventricle after exposure of heart. Serum was analyzed for total protein by micro biuret method with pure bovine serum albumin serving as standard(8). Duplicate 0.1 ml samples of the serum were then subjected to paper electrophoresis using a Durrum type cell (Spinco commercial model R). After 20 hours at 5 ma. in a 0.05 M veronal buffer the electrophoretic strips were dried, stained with brom phenol blue and analyzed using the

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