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Research Articles

Synthesis and Pharmacological Studies of Some Aliphatic Hemicholinium Analogs

By MARVIN F. POWERS, STEFAN KRUGER, and FRED W. SCHUELER

The aromatic nucleus of hemicholinium-3 was replaced with an aliphatic hexamethylene chain without loss of the characteristic pharmacological activity. The toxic dose was elevated in mice about tenfold by the change. Choline chloride was a very effective antidote to intoxication from the aliphatic hemicholinium. Another compound synthesized, a pyridine analog, had marked anticholinesterase activity, but caused a flaccid paralysis in avian musculature. A third compound, a trimethylamine analog, elicited neuromuscular blockade typical of decamethonium.

HEMICHOLINIUM-3, hereafter called HC-3, was synthesized in 1954 during an investigation of a series of bis-quaternary ammonium compounds (1). The general pharmacology of HC-3 has recently been reviewed by Schueler (2). Marshall and Long (3) synthesized and investigated pharmacologically some hemicholinium analogs and found none to be more potent than HC-3. The analogs studied by these workers contained structural changes at the cationic heads and in the biphenyl nucleus. It appears that the crucial moiety is at the cationic head rather than at the biphenyl grouping, since the introduction of an ether or a methylene linkage between the two phenyls elicited only a slight decrease in potency, whereas certain relatively minor changes at the cationic heads either markedly reduced or abolished HC-3-like activity (3). Considering the alterations already studied, one may question whether the aromatic nucleus is an essential feature in the HC-3 molecule. The introduction of a methylene chain would not only incorporate an aliphatic moiety, but would also provide an approach for a systematic study of the effect on pharmacologic activity

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of alteration of the distance between the cationic heads, provided an aliphatic chain can successfully replace the biphenyl nucleus, with retention of characteristic hemicholinium-like action.

The aliphatic moiety least likely to alter extended molecular length would seem to be a hexamethylene chain, since in its extended configuration this chain approximates very closely the length of the biphenyl nucleus. The aromatic nucleus is rigid and permits no variation of distance between cationic heads, whereas the methylene chain allows a great deal of folding. However, the presence of two positively charged atoms in this molecule would necessarily entail coulombic repulsion between the like charges and thus tend to cause the molecule to be in a fully extended configuration. This paper reports the pharmacological studies of some aliphatic HC-3 analogs containing a hexamethylene chain.

MATERIALS AND METHODS

Suberic acid (0.1 mole) was reacted with 0.3 mole of thionyl chloride at 50° for 4 hours in a flask fitted with a reflux condenser (4). The excess thionyl chloride was then evaporated under reduced pressure. The acid halide was added dropwise to an ethereal solution of diazomethane at 0°. The diazomethane was prepared from Diazald[1] and dried over potassium hydroxide for 30 minutes. The reaction mixture was allowed to stand at room temperature overnight. The precipitated bis-diazoketone was then dissolved in chloroform, and anhydrous hydrochloric acid was bubbled through the solution for 20 minutes. Evaporation of the solvent left the crude compound 1,6-bis(chloroacetyl)-*n*-hexane as a brown mass.

After four recrystallizations from aqueous methanol, the product was obtained in the form of white plates melting at 85°, as stated in the literature (4), with a 60% yield.

One gram of the 1,6-bis(chloroacetyl)-*n*-hexane was dissolved in 15 ml. of cold dioxane, mixed with an excess (2 ml.) of β-dimethylamino ethanol, and heated on a water bath for 30 minutes. The gummy material which formed was dissolved in absolute alcohol. Upon addition of ether a white solid separated out. Three recrystallizations gave a product melting at 212°, with decomposition. This product, 1,10-bis(dimethyl-β-hydroxyethylammonium)-*n*-decan-2,9-dione dichloride was designated P-16-A.

The pyridine compound, P-16-B, was prepared in a similar manner, using pyridine as the amine. This product melted at 216°, with decomposition.

P-16-C was prepared by reacting trimethylamine with the 1,6-bis(chloroacetyl)-*n*-hexane in a pressure bottle for 2 hours at 100°. The product melted at 225°, with decomposition.

The melting points in all cases involving decomposition were determined by placing the compound on a hot Fisher-Johns melting block heated to within a few degrees of the expected melting point. Analyses of these compounds are detailed in Table I. Figure 1 shows the molecular configurations of these compounds and also that of HC-3.

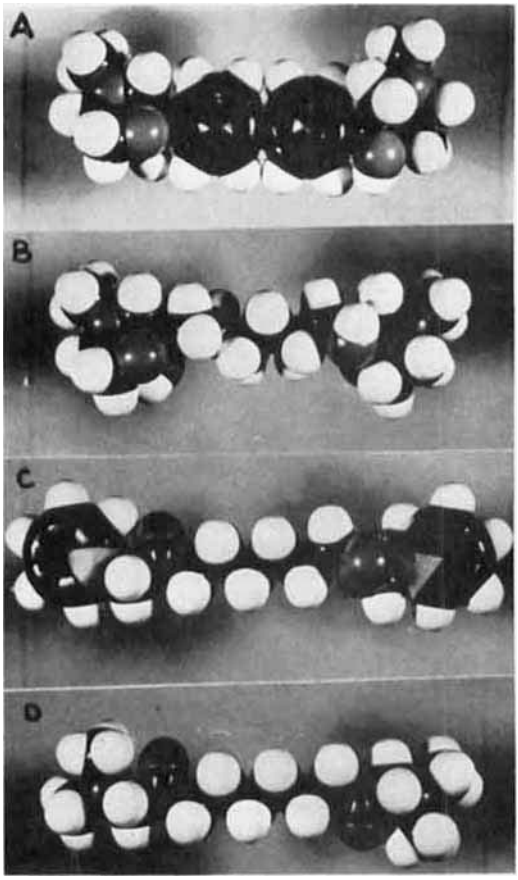

Fig. 1.—It can be seen that internitrogen distances are similar when the molecules are maximally extended. A is HC-3, B is P-16-A, C is P-16-B, and D is P-16-C.

Animal Studies.—Toxicity studies were done on white Swiss mice weighing about 25 Gm. In each case the compound was dissolved in 0.9% sodium chloride and injected intraperitoneally. Each dose level was administered to a group of 10 mice. Toxicity data were calculated by the method of Litchfield and Wilcoxon (5). Drug antagonism studies were carried out by administering various drugs in conjunction with each of the compounds being investigated, as specified in Table II.

Studies on blood pressure, sciatic-gastrocnemius activity, and respiratory function were done on nine mongrel dogs anesthetized with sodium pentobarbital 35 mg./Kg. intravenously. The trachea was cannulated and a water manometer was attached for recording respiration on smoked kymograph paper. The left common carotid artery was cannulated and blood pressure recorded by using a mercury manometer. Five milliliters of 2% mepesulfate (Roche) was introduced into the carotid cannula to avoid clotting of blood. The right vagus nerve was isolated and sectioned, and periodically the periph-

[1] Aldrich Chemical Co.

TABLE I.—TOXICITY, MELTING POINT, AND ANALYSIS OF COMPOUNDS

Compound	M. P., °C.	LD_{50} in Mice, mg./Kg.	Elemental Analysis, % Required C	Required H	Required N	Found C	Found H	Found N
P-16-A $C_{18}H_{38}Cl_2N_2O_4$	212	0.5 (0.38–0.65)	51.7	9.1	6.7	51.6	9.0	6.7
P-16-B $C_{26}H_{26}Cl_2N_2O_2$	216	10.0 (6.7–13.0)	59.1	6.7	6.8	59.3	6.8	6.5
P-16-C $C_{16}H_{34}Cl_2N_2O_2$	225	6.5 (5.8–7.2)	53.7	9.5	7.8	53.5	9.2	7.5

TABLE II.—PHARMACOLOGICAL TESTS OF COMPOUNDS

Situation	No. Mice	Compound	Dose mg./Kg.	Route	Compd. Used Jointly	Dose, mg./Kg.	Route	Deaths
1	10	P-16-A	0.5	i.p.	....	..	...	9
2	10	P-16-A	0.5	i.p.	Choline	20	s.c.	2
3	10	P-16-A	1.0	i.p.	Choline	20	s.c.	9
4	10	P-16-A	1.0	i.p.	Choline	40	s.c.	5
5	10	P-16-B	12.0	i.p.	....	..	...	9
6	10	P-16-B	12.0	i.p.	Choline	20	s.c.	8
7	10	P-16-B	12.0	i.p.	Atropine	2.0	s.c.	7
8	10	P-16-C	7.0	i.p.	....	..	...	10
9	10	P-16-C	7.0	i.p.	Choline	20	s.c.	8
10	10	...	..	..	Choline	40	s.c.	0

Chi-Square

Situation	P
1 and 2	<0.01
3 and 4	>0.10
4 and 5	>0.50

eral stump was electrically stimulated. The right sciatic nerve was isolated, ligated tightly, and arranged for supramaximal peripheral stimulation with a Grass model S4C stimulator at specified frequencies by shielded silver electrodes. The gastrocnemius was also isolated, sectioned distally, and connected to a lever for kymographic recording. Test doses of histamine, epinephrine, and acetylcholine were administered several times to obtain a standard response. The vagus was stimulated, and the intact right carotid artery was temporarily occluded for additional standard responses. This testing operation was conducted periodically after administration of P-16-A, P-16-B, and P-16-C to screen for autonomic-cardiovascular effects.

Bilateral sciatic-gastrocnemius preparations were done on 12 adult albino rabbits anesthetized with 200 mg./Kg. of phenobarbital sodium administered intravenously 1 hour prior to the experiment. Both sciatic nerves were tightly ligated and individually arranged for supramaximal peripheral stimulation by a Grass stimulator at various frequencies, using shielded silver electrodes. The gastrocnemius muscles were attached to kymographic levers for recording contraction height.

Seven chicks, four weeks old, were injected intramuscularly with 1 or 2 mg. of compound and observed for evidence of flaccid or spastic paralysis.

Twelve adult white leghorn hens were anesthetized with 25 mg./Kg. of sodium pentobarbital and prepared for kymographic recording by isolating the sciatic nerve, ligating it, and arranging it for peripheral stimulation supramaximally at specified frequencies at 5 msec. duration. The gastrocnemius muscle was attached to a kymograph lever. The cannulated trachea was insufflated with oxygen.

Enzyme Studies.—Enzyme inhibition studies were carried out manometrically, using "true" acetylcholinesterase, a commercial purified preparation of bovine erythrocyte acetylcholinesterase.[2] For the competitive studies, "pseudo" cholinesterase was used; the source was fresh dog plasma diluted 1:5 with 0.9% sodium chloride. Flasks contained a total volume of 2.0 ml. for each experiment. The standard procedure of Ammon was used as modified by Augustinsson (6).

Acetylcholine was used as the substrate, and a bicarbonate buffer served as the medium. All appropriate flask readings were corrected for nonenzymatic hydrolysis and for atmospheric pressure variations. Readings were taken every 10 minutes for a period of 1 hour in the experiments using "true" esterase, and every 5 minutes for a 40-minute period in the "pseudo" esterase experiments. Results were expressed as μl. of carbon dioxide evolved per interval of time. Determinations were always carried out using duplicate flasks.

RESULTS AND DISCUSSION

The toxicity of the aliphatic hemicholinium, P-16-A, is not as great as that of HC-3, the former being about one-tenth as potent in mice. See Table I. This decrease might be explained if one assumes that not all the molecules are maximally extended and that complete extension is essential for optimal activity. Similarly, the toxicities of the pyridine and trimethylamine analogs were decreased by about the same degree.

Figure 2 shows a typical result of P-16-A on a

[2] Winthrop Laboratories.

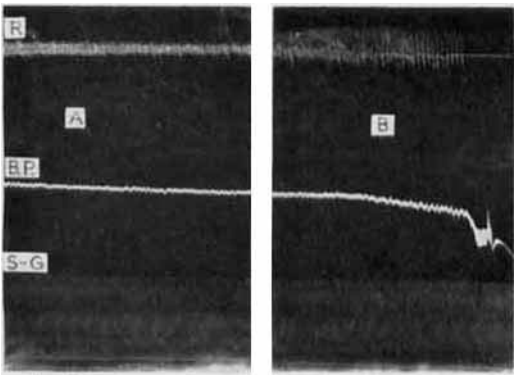

Fig. 2.—A recording of dog respiration (R), blood pressure (B.P.), and sciatic-gastrocnemius (S-G) effects when P-16-A, 1 mg./Kg. is administered at A. Respiratory function failed at B, 105 minutes after A, whereas the S-G activity continued.

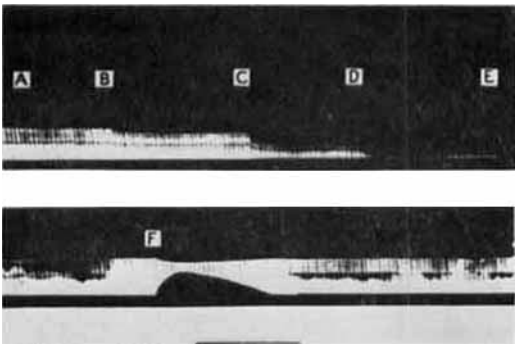

Fig. 3.—Adult hen sciatic-gastrocnemius preparation and the effects of P-16-B (top record) given in graded doses. A = 0.5 mg./Kg.; B = 1.0 mg./Kg.; C = 1.25 mg./Kg.; D = 1.5 mg./Kg.; E = 2.00 mg./Kg. No contracture is evident even though P-16-B is a cholinesterase inhibitor in mammals. Bottom record is P-16-C 80 mcg./Kg. given at F.

dog. The neuromuscular responses continue unaltered when stimulation is one per 10 seconds. However, the respiration fails about 100 minutes after administration. This latent action is very typical of HC-3 poisoning. Blood pressure falls only after respiratory embarrassment and failure have occurred. If oxygen is insufflated intratracheally throughout respiratory paralysis, the blood pressure remains stable, and eventually the respiratory function is restored. Administration of choline chloride intravenously, 20 mg./Kg., antagonizes the respiratory paralysis.

In a rabbit sciatic-gastrocnemius preparation P-16-A mimics HC-3. Frequency of stimulation is an important factor, since a frequency rate of one per 10 seconds is never accompanied by failure or by depression of function in the dosage used. In contrast, the rate of one per second is accompanied by functional failure. It is indeed true that an increase of frequency of stimulation can lower the effective dose of a neuromuscular blocking agent such as curare and decamethonium (7). However, with the latter two, it was observed that onset was rapid.

The trimethylamine analog (P-16-C) did not produce an HC-3-like response. The onset of paralysis was rapid and was not antidoted by choline chloride, as was P-16-A. See Table II. The functional response of the nerve-muscle preparation was actually further depressed by choline chloride. The hen sciatic-gastrocnemius preparation exhibited a mixed response with depolarization of end-plates, as reflected by contracture, and a slight flaccid paralysis, as represented by the decrease in contraction height. See Fig. 3.

The pyridine analog, P-16-B, exhibited effects which were very interesting. When 1 mg./Kg. was administered to dogs, a transient fall in blood pressure was noted; when a test dose of acetylcholine was given, the recovery of blood pressure was delayed. Increasing the dose of P-16-B to 5 mg./Kg. caused neuromuscular paralysis. Both doses caused copious salivation, micturition, and defecation. These latter effects are also observed in the intact, unanesthetized rabbit. Even though the electrical stimulus was predetermined to have been supramaximal, a transient increase in contraction height was observed.

A similar effect is observed with well-known cholinesterase inhibitors such as eserine. When the present compounds were studied in the presence of cholinesterase, it was found that P-16-B was the most potent *in vitro* inhibitor of the three compounds synthesized (see Table III). Assuming that cholinesterase inhibition was the primary action of P-16-B, it would be expected that the compound would produce depolarization and spastic paralysis in the chicken. The actual effect observed is, however, a flaccid paralysis (see Fig. 4). That P-16-B does not produce even transient spasticity is demonstrated in Fig. 4. When one starts with a very small dose P-16-B (1 mcg./Kg.) and gradually increases the dosage in a hen sciatic-gastrocnemius preparation, there is no apparent effect until a dose of 1 mg./Kg. is given. This latter dose causes a decrease in contraction height with no evidence of contracture. Table III illustrates the type of enzyme inhibition that P-16-B seems to produce. It was found that inhibition is apparently competitive in nature. Plotting the reciprocal of activity at several concentrations of the inhibitor (P-16-B) against the reciprocal of substrate concentration yields "curves" which share the same origin on the ordinate, thereby

TABLE III.—STUDIES OF CHOLINESTERASE ACTIVITY BY MANOMETRIC METHODS[a]

Acetylcholinesterase from Bovine Erythrocytes, Concentration		
Compound	Moles	% Inhibition
HC-3	1×10^{-3}	25 (1)[b]
P-16-A	1×10^{-3}	68 (1)
	1×10^{-4}	19 (2)
P-16-B	1×10^{-3}	97 (1)
	1×10^{-4}	93 (1)
	1×10^{-5}	78 (1)
	1×10^{-6}	33 (1)
P-16-C	1×10^{-3}	53 (1)
	1×10^{-4}	29 (1)
Eserine	1×10^{-3}	96 (1)
	1×10^{-4}	96 (1)
	1×10^{-5}	91 (1)
	1×10^{-6}	67 (1)

[a] Acetylcholine concentration, 1×10^{-3} *M*.
[b] Figures in parentheses represent number of determinations using duplicate flasks.

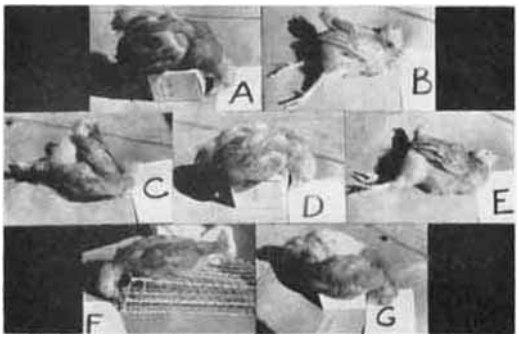

Fig. 4.—Four-weeks old chicks injected intramuscularly with the corresponding compound. Dosage was 1 mg. total dose for each. A = Curare; B = decamethonium; C = succinylcholine; D = HC-3; E = P-16-C; F = P-16-B; G = P-16-A. All compounds caused rapid paralysis within 5 minutes except HC-3 (D) and P-16-A (G) which produced paralysis after a latent period of 20 to 30 minutes.

resembling the Lineweaver-Burk plot of a competitive inhibitor.

The fact that P-16-B, a potent anticholinesterase agent, effects a flaccid rather than a spastic type of paralysis in avian muscle is particularly interesting. A possible explanation may follow from the concepts of Ariëns, Van Rossum, and Simonis (8). P-16-B may represent a product with very high affinity but low intrinsic or specific activity with respect to postsynaptic acetylcholine receptors at the neuromuscular junction. Thus, while P-16-B is a potent anticholinesterase drug, its high affinity (in the manner of curare) causes it to block the actions of excess acetylcholine at this junction. For muscarinic receptors P-16-B apparently does not exhibit such a high affinity and therefore the effects of excessive acetylcholine due to its anticholinesterase effects are observed.

SUMMARY

Three new substances related to hemicholinium were synthesized: 1,10-bis(dimethyl-β-hydroxyethyl ammonium)-*n*-decane-2,9-dione dichloride, 1,10-bis(pyridinium)-*n*-decane-2,9-dione dichloride, and 1,10-bis(trimethylammonium)-*n*-decane-2,9-dione dichloride.

Pharmacological studies indicated that the biphenyl nucleus of HC-3 can be replaced with an aliphatic chain without serious loss of HC-3-type activity as observed by the actions which P-16-A produced. Possible reasons for the decrease in toxicity of the compounds have been discussed.

One of the compounds, P-16-B, was an anticholinesterase agent nearly as potent as eserine. Paradoxically, this compound prevented endplate depolarization. A possible explanation concerning the flaccid paralysis is discussed.

P-16-C appeared to exert a mixed action by causing contracture paralysis (spasticity), accompanied by a slight decrease in contraction height in a chicken sciatic-gastrocnemius preparation.

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