Anal. Calcd. for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.83; N, 6.33. Found: C, 64.71; H, 6.68; N, 6.25.

2-(3′,4′-Dimethoxybenzyl)lactic Acid (9).—To 47.7 g. of 2-(3′,4′-dimethoxybenzyl)lactonitrile (**8**) was added 108 ml. of concd. hydrochloric acid. The mixture was refluxed for 5 hr., concentrated, 20 ml. of water added and the mixture again concentrated. The residue was cooled to room temperature and extracted with ethyl acetate. The extract was washed with water, slurried with 1 g. of decolorizing charcoal, filtered and concentrated *in vacuo* to a small liquid volume containing crystals. The mixture was diluted with 100 ml. of ether and allowed to stand for 16 hr. Filtration yielded 24.84 g. (49.5%) of product, m.p. 118–121°; $\lambda_{max}^{CH_3OH}$ 230 mμ, (ε = 8,150), 278 mμ, (ε = 2,690), sh 283 mμ, (ε = 2,450).

The analytical material was obtained by recrystallization from acetone–Skellysolve B, m.p. 114–116°; $\lambda_{max}^{CH_3OH}$ 229.5 mμ, (ε = 8,080) 277.5 mμ, (ε = 2,740), sh 283 mμ, (ε = 2,475); $\lambda\lambda_{max}^{Nujol}$ 2.90, 3.6–3.9, 5.79, 6.18, 6.25 and 6.56 μ.

Anal. Calcd. for $C_{12}H_{16}O_5$: C, 59.99; H, 6.71. Found: C, 59.88; H, 6.40.

2-Acetoxy-2-(3′,4′-diacetoxybenzyl)propionic Acid (11).—To 13.85 g. of 2-(3′,4′-dimethoxybenzyl)lactic acid (**9**) was added 78.5 ml. of 48% hydrobromic acid. The mixture was purged with nitrogen, refluxed for 2 hr. and concentrated *in vacuo*. The residue was dissolved in *tert*-butyl alcohol and the mixture concentrated to dryness *in vacuo*. This procedure was repeated. The dark residue of crude 2-(3′,4′-dihydroxybenzyl)lactic acid (**10**) was dissolved in 65 ml. of pyridine and, while the flask was immersed in a cooling bath to maintain the temperature between 10 and 20°, 65 ml. of acetic anhydride was added. The mixture was allowed to stand for 16 hr. at room temperature and then concentrated *in vacuo* to an amber gum. This gum was dissolved in ethyl acetate and extracted successively with *N* hydrochloric acid, water and saturated salt solution. The ester phase was dried over anhydrous magnesium sulfate, concentrated *in vacuo* and the residue crystallized from a (1:1) benzene–hexane mixture. The crude product had m.p. 120–123°; $\lambda_{max}^{CH_3OH}$ 274 mμ, (ε = 6,050) sh 270 mμ, (ε = 5,380). The yield amounted to 18.28 g. Repeated recrystallizations from benzene yielded a product (11), m.p. 124–126°; $\lambda_{max}^{CH_3OH}$ 264 mμ, (ε = 5,380), sh 269 mμ, (ε = 4,970); $\lambda\lambda_{max}^{Nujol}$ 3.8–4.0, 5.70–5.75, 5.85, 6.25 and 6.61 μ.

Anal. Calcd. for $C_{16}H_{18}O_8$: C, 56.80; H, 5.36; acetyl, 38.2. Found: C, 56.72; H, 5.66; acetyl, 41.7.

DL-2-(3′,4′-Dihydroxybenzyl)lactic acid (10).—A mixture of 7.31 g. of 2-acetoxy-2-(3′,4′-diacetoxybenzyl)propionic acid (11), 86.3 ml. of 2.5 *N* hydrochloric acid and 30 ml. of water was purged with nitrogen and refluxed under a nitrogen atmosphere for 2 hr. The resulting mixture was concentrated *in vacuo*, and extracted with ether. The ethereal extract was washed with water and concentrated to an oil, which on drying *in vacuo* at 100° for 1 hr. yielded 4.45 g. (95.0%) of DL-2-(3′,4′-dihydroxybenzyl)lactic acid (**10**) as an amorphous solid, $\lambda_{max}^{CH_3OH}$ 282 mμ (ε = 5,980); $\lambda\lambda_{max}^{pyridine}$ 3.1–4.1 multiple absorption, 5.85, 6.59 μ. The infrared spectrum was the same as that of the acid before acetylation and unlike that of the 2,3′,4′-triacetate (11).

Anal. Calcd. for $C_{10}H_{12}O_5$: C, 56.60; H, 5.70. Found: C, 56.64; H, 5.97.

Acknowledgment.—The authors are indebted to Messrs. R. W. Walker, A. Kolowsky, and B. H. Arison, respectively, for the infrared, ultraviolet, and n.m.r. data reported; to Mr. R. N. Boos and associates for microanalytical data, and to Mr. A. J. Davidson for technical assistance. We also are indebted to Drs. C. A. Stone, L. S. Watson, C. C. Porter, and R. W. Schayer of the Merck Institute for Therapeutic Research for the biological results reported.

Structure–Action Relations in N,N-Dimethyl-2-halogenophenethylamines

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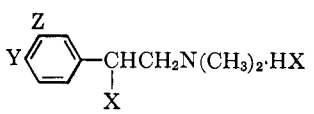
Antagonism to epinephrine, norepinephrine and histamine has been investigated in a series of compounds related to N,N-dimethyl-2-halogenophenethylamine. The relation between structure and antagonism is demonstrated and the implications for the intermediary chemical species responsible in aqueous reaction mixtures is discussed. Antagonism to stimulation of oxyntic cells and inhibition of rat uterus by histamine is noted.

Hunt[1] first reported the antiepinephrine activity of N,N-dimethyl-2-chlorophenethylamine (DMEA) and Ferguson and Wescoe[2] showed that this compound possessed in addition muscarine-like, nicotine-like and relaxant properties. Graham and James[3] confirmed these findings and examined some 60 analogs. Antagonism to epinephrine, norepinephrine, histamine, and 5-hydroxtryptamine was demonstrated. Three structural requirements were found to be necessary for antiepinephrine activity, *viz.*, (1) an aromatic ring structure, (2) a 2-halogenoethyl group, and (3) a secondary or a tertiary amino group. The ethanolamine derived from DMEA is not active against epinephrine, norepinephrine, histamine or 5-hydroxytryptamine but is a powerful local anaesthetic.

The present report is concerned with exploration of the substituent in the 2-position, on the phenolic ring, and on the 2-carbon. There are three groups of compounds in this series, with varying structures



Substituents on the ring: (1) *Monosubstituted compounds* where Z (*meta*) in the formula is H, the other substituent being Cl, Br, I, F, CH_3, or C_6H_5; compounds where Y (*para*) is H and Z is Cl, Br, or CH_3; in all cases but one (Table I, 13) X being Br. (2) *Disubstituted compounds* where X is Br or Cl and Y and Z are either dichloro, dibromo or dimethyl, or combinations of Cl, Br, CH_3, and F. (3) *Ethanolamines* where one hydrogen atom of the carbon in position 2 in the ethylamine side chain has been replaced by OH. The substituents on the phenolic ring are, respectively, dibromo, dichloro, dimethyl, and F–Br. These are the hydrolysis products of selected members of the disubstituted compounds. The structures are shown in Table I. Compound 29 has a structural resemblance to dichloroisoproterenol

(1) C. C. Hunt, *J. Pharmacol. Exptl. Therap.*, **95**, 177 (1949).
(2) F. C. Ferguson and W. C. Wescoe, *ibid.*, **100**, 100 (1950).
(3) J. D. P. Graham and G. W. L. James, *J. Med. Pharm. Chem.*, **3**, 489 (1961).

TABLE I

The structures of the compounds, the antagonism to the pressor action of epinephrine and norepinephrine (1.0 μg./kg. i.v.) in the spinal rat, as moles × 10^{-9}/kg. wt.; and the antagonism to the spasmogenic effect of histamine (2 μg./l.) on the isolated ileum of the guinea pig, as μmoles/l.

No.	Y	Z	X	ED_{50} on rat blood pressure in moles. × 10^{-9}/kg. wt. Epinephrine (1)	Norepinephrine (2)	ED_{50} on isolated guinea pig ileum in μmoles/l. Histamine (3)
			Group 1. Monosubstituted			
1	H	H	Br	35.00 ± 7.0	56.00 ± 7.1	3.3
2	F	H	Br	7.00 ± 2.0	7.40 ± 2.0	20
3	Cl	H	Br	2.10 ± 0.3	2.10 ± 0.3	6.4
4	Br	H	Br	1.30 ± 0.20	1.30 ± 0.22	3.7
5	I	H	Br	0.56 ± 0.05	0.51 ± 0.05	2.2
6	Me	H	Br	0.50 ± 0.05	0.53 ± 0.05	25
7	Ph	H	Br	30.00 ± 4.1	30.00 ± 4.0	
8	H	F	Br	30.00 ± 6.0	32.00 ± 6.4	3.7
9	H	Cl	Br	7.00 ± 1.4	7.10 ± 1.4	4
10	H	Br	Br	5.00 ± 1.2	5.00 ± 1.2	2.6
11	H	I	Br	4.00 ± 0.9	4.00 ± 0.9	1.3
12	H	Me	Br	3.5 ± 0.9	3.00 ± 0.9	
13	Cl	H	Cl	.....	.....	3.0
			Group 2. Disubstituted			
14	F	Cl	Br	6.40 ± 1.1	6.40 ± 1.0	
15	F	Br	Br	2.70 ± 0.4	2.70 ± 0.4	0.9
16	F	Me	Br	1.50 ± 0.3	1.50 ± 0.3	
17	Cl	Cl	Cl	2.80 ± 0.3	2.80 ± 0.3	0.07
18	Cl	Cl	Br	1.30 ± 0.2	1.30 ± 0.2	1.0
19	Cl	Br	Br	1.20 ± 0.06	1.20 ± 0.06	0.5
20	Cl	Me	Br	1.10 ± 0.07	1.10 ± 0.07	
21	Br	Cl	Br	1.00 ± 0.1	1.00 ± 0.1	0.7
22	Br	Br	Cl	0.9 ± 0.1	0.9 ± 0.09	.08
23	Br	Br	Br	0.45 ± 0.02	0.50 ± 0.02	.1
24	Br	Me	Br	0.6 ± 0.07	0.60 ± 0.07	
25	Me	Me	Cl	1.00 ± 0.1	1.00 ± 0.1	0.9
26	Me	Me	Br	0.5 ± 0.04	0.5 ± 0.04	1.2
27	Me	Br	Br	0.30 ± 0.01	0.30 ± 0.01	
			Group 3. Ethanolamines			
28	F	Br	OH	1000[a]	1000[a]	0.81
29	Cl	Cl	OH	1000[a]	1000[a]	0.09
30	Br	Br	OH	1000[a]	1000[a]	0.14
31	Me	Me	OH	1000[a]	1000[a]	0.7
32	H	H	OH	1000[a]	1000[a]	25,000[a]
33	Cl	Cl	OH etc.	inactive		0.32
			Reference compound			
34	2-Bromo-N-ethyl-N-1-(naphthylmethyl)ethylamine					

[a] Inactive at the dose stated.

(D.C.I.)[4] which is a competitive blocker of the β-receptors for catecholamine, and all of the ethanolamines resemble sympathomimetic amines. A brief notice of some members of this series has appeared.[3,5]

The compounds of Groups 1 and 2 are crystalline substances. Some are readily soluble in water, all in acid alcohol or acetone from which dilutions with water may be made. In neutral or just alkaline aqueous solution they are unstable, *e.g.*, a 0.02 *M* solution of compound 17 in acetone and ion-free water (1:1) at 37° releases 94% of its halogen almost instantly, presumably with cyclization to ethyleneiminium ion (E^+) as indicated by the presence of a thiol-consuming species. Fall of pH due to release of combined acid begins at once and builds up for 4 hr., by which time E^+ is absent and free halogen is 100%. Hydrolysis to the corresponding ethanolamines proceeds at a variable rate, which is greatly accelerated by excess of alkali. Details of synthesis and quantitative studies of the purely chemical behavior of the parent compounds and E^+ have been published in a thesis. All compounds were administered within 2 min. of solution in every application. The ethanolamines (Group 3) are oily bases, soluble in dilute acetic acid, from which they were

TABLE II

The pA_x values are stated for 2 and 14 min. contact with 34 and 23 on rabbit uterus tested with epinephrine: the pA_x values for 34, 17, its ethanolamine 29, and pyrilamine with guinea pig ileum tested with histamine.

	Antiepinephrine		Antihistamine			
$pA_x - t$	34	23	34	17	29	Pyrilamine
pA_2–2	6.3	7.6	8.9	6.0	6.3	8.8
pA_2–14	7.0	7.8	9.0	6.6	6.5	9.4
pA_{10}–2	7.0	7.0	8.8	5.3	5.6	8.0
pA_{10}–14	7.2	7.0	9.0	5.6	5.9	8.5

(4) B. Levy and R. P. Ahlquist, *J. Pharmacol. Exptl. Therap.*, **130**, 334 (1960).

(5) J. D. P. Graham and M. A. Karrar, *Biochem. Pharmacol.*, **8**, 491 (1961).

TABLE III

Comparison of the effect of compounds 17 and 29 with D.C.I. on "β receptors" of catecholamine and of compounds 23 and 30 on selected actions of histamine: + denotes blockage, − denotes failure to block.

	Catecholamine				Histamine		
	17	29	D.C.I.		23	30	Pyrilamine
Rabbit heart	−	−	+	Guinea pig ileum	+	+	+
Guinea pig atrium	−	−	+	Guinea pig bronchus	+	+	+
Rabbit gut	+	+	±	Guinea pig gastric HCl	+	+	−
Guinea pig peristalsis	−	−	±	Cat gastric HCl	+	+	−
Cat uterus (nonpregnant)	−	+	+	Dog gastric HCl	+	+	−
Cat B.Pr. (isoproterenol)	−	±	+	Rat-"Shay"	−	−	−
				Rat-uterus	+	+	−

administered after dilution and buffering in Krebs solution. Compound 29 was available as a water-soluble crystalline hydrochloride.

Experimental

Antagonism to the Actions of Epinephrine, Norepinephrine, and Isoproterenol.—The ED_{50}'s for antagonism of the pressor action of 1.0 μg./kg. or 5.4×10^{-9} mole of base/kg. (as salt) of epinephrine and norepinephrine in the spinal rat[3] are expressed as moles $\times 10^{-9}$ of compound/kg. rat in Table I. Injections of the compounds were made every 30 min. and considered to be additive because the duration of an established blockade in rats is 1–2 hr. Details[3] of 1, in which the phenyl is unsubstituted and the halogen on C-2 is Br, and of the ethanolamine from it (32) are included. These compounds are very potent; *e.g.*, 2-bromo-N,N-dimethyl-2-(4-methyl-3-bromophenethyl)amine has an ED_{50} in the rat pressor test of 0.3×10^{-9} mole/kg. and 2-bromo-N,N-dimethyl-2-(3-iodophen)ethyleneiminium ion, available as picryl sulfonate, is twice as active as its parent compound (11) in this test. The E^+ of 18 and 23 are also active. The ethanolamines (28–33) do not antagonize the pressor response to epinephrine and norepinephrine. The product of hydrolysis of the parent compound, cannot therefore be the active chemical species for this effect, but E^+ may be. Compounds 1–27 cause a rise in blood pressure when first injected intravenously in rats or cats, an effect which becomes progressively less and is abolished at the same time as the pressor response to norepinephrine.[6] Antagonism is manifest less than 3 min. after injection and its duration is short (50–90 min. in anesthetized cats and 1–2 hr. in spinal rats). *The blockade exerted by these compounds is surmountable*, a tenfold increase in the challenging dose of epinephrine restoring the initial response in rats in which it had been abolished. This relation contrasts with that exerted by Dibenamine. When 2-chloro-2-dibromophenyl-N,N-dimethylamine (22) is dissolved in distilled water at 37° and sufficient NaOH added to neutralize evolved acid, the antiepinephrine potency of the solution falls off exponentially with time,[7] and is extinguished after 4 hr. (see Fig. 1).

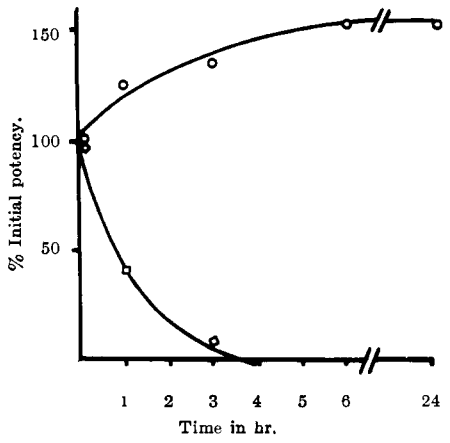


Fig. 1.—The relation between antiepinephrine and antihistamine potency of a neutralized aqueous solution of compound 23 kept at 37° and examined over 24 hr.

When isolated rabbit uterus is mounted in a Ringer's solution with low Ca^{++} content it displays little spontaneous activity and may be used to determine pA_x values[8] for antagonists. The pA_x value is the negative log of the *M* concentration of antagonist which will reduce the effect of a multiple dose (x) of agonist to that of a single dose, on a stated tissue and with a stated duration of contact (*t*). The higher the index the more active is the antagonist. These are given in Table II for 23. This compound, which is approximately 10,000 times stronger than the reference compound, 2-bromoethyl-N-ethyl-(naphthylmethyl)ethylamine,[9] in antagonizing the pressor response of the spinal rat to epinephrine is only 10 times more potent in the rabbit uterus test when the challenging dose of epinephrine is doubled (pA_2) and no more potent when it is increased tenfold (pA_{10}).

The structural resemblance between 29 and dichloroisoproterenol (D.C.I.) (33) has been pointed out. Comparison of the effects in blocking β-receptors to epinephrine in a series of preparations showed that D.C.I. is by far the more potent of the two (see Table III).

Antagonism to Histamine.—All the compounds antagonize the spasmogenic action of histamine on isolated guinea pig ileum. The ED_{50}'s are stated in Table I in μmoles/l. of bath fluid. With the exception of the ethanolamines (28–32) and the 2-chloro compounds (17, 22, 25) they are spasmogenic in the concentrations used and the tests have to be done in the presence of 1 μg./l. of atropine sulfate which may increase their apparent potency. The strongest are weaker than pyrilamine, *e.g.*, 17 or 22 has an ED_{50} of approximately 0.07 μmole/l. in this test and a $pA_2 - 2$ min. of 6.0, whereas pyrilamine has an ED_{50} of approximately 10^{-6} μmole/l. and a $pA_2 - 2$ min. of 8.8. The reference compound 34 is similar in potency to pyrilamine.[9] The pA_x values[8] of selected compounds were measured after 2 and 14 min. contact with guinea pig ileum stimulated with histamine 10^{-3} μmole/l. They are listed in Table II which shows that 17 increases in potency with increasing time of contact with the tissue, as does pyrilamine. At all dose levels tested it is always possible to overcome the antagonism exerted by compound 17 and by pyrilamine. If the dose of histamine is increased a greater amount of either is needed to restore the response to the initial level ($pA_{10} < pA_2$) whereas this is a feature of the action of 34 over a limited range only. The relation log dose antagonist to log dose histamine needed to restore the initial level of contraction is a linear one over 4 log places for histamine and 3 log places for 17. The antagonism may therefore be described as "competitive." The dose-response curve to histamine is shifted to the right by determining it in the presence of increasing amounts of pyrilamine or of 17 and it becomes less steep. Over a range of 2 log places tested (10 μg.–1.0 mg. of 17 added), the blockade is fully surmountable. This relation is illustrated in Fig. 2. The onset of action of 17, pyrilamine and 34 is rapid. The antagonism exerted by pyrilamine in concentrations over a range of 3 log places and of 17 in concentrations over a range of 2 log places (which are fully effective) are removed by washing the tissue, unlike that of 34, which is only removed in part if and when the concentration exceeds a low threshold value. The asymptote of the response–time curve is thus not permanently lowered by 17 nor by pyrilamine but is by the classical 2-halogenoalkylamine above a threshold concentration. The rate of recovery from pyrilamine is fast (10–30 min.), from 17 is slow (20 min. to 4 hr.). This relation is displayed in Fig. 3.

Compound 17 therefore lies somewhere between the blockade exerted by antihistamines and 2-halogenoalkylamines.[10,11] The

(6) J. D. P. Graham, *Brit. J. Pharmacol.*, **16**, 77 (1961).
(7) J. D. P. Graham, *ibid.*, **12**, 489 (1957).
(8) H. O. Schild, *ibid.*, **2**, 189 (1947).
(9) J. D. P. Graham and G. P. Lewis, *ibid.*, **8**, 54 (1953).
(10) M. Nickerson, *Pharmacol. Rev.*, **9**, 246 (1957).
(11) J. D. P. Graham, *J. Med. Pharm. Chem.*, **2**, 499 (1960).

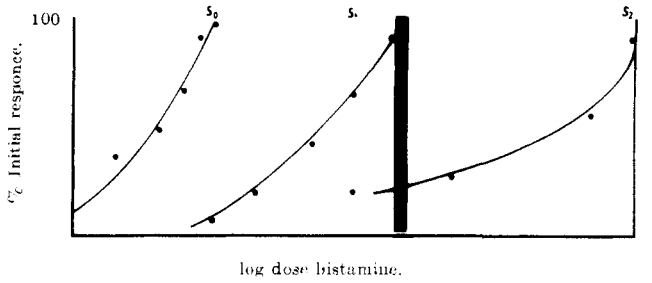


Fig. 2.—The dose-response curve to histamine on guinea pig ileum is shifted to the right 3 log places (the black bar symbolizes one log place omitted for convenience) by increasing the dose of antagonist (compound 17) from an effective dose (S_0) by 10 (S_1) and by 100 (S_2). The slope becomes less steep but the antagonism is always surmountable.

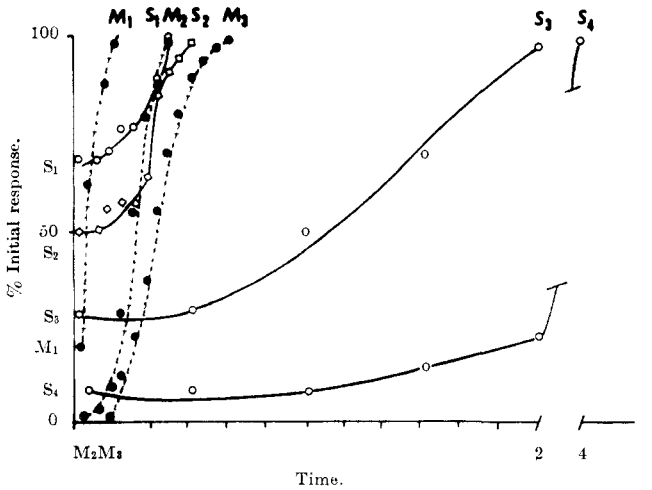


Fig. 3.—Antagonism of pyrilamine and 17 to histamine on the isolated guinea pig ileum. The relation of dose of antagonist in contact for a fixed time to rate and degree of recovery from it is shown. Broken lines symbolize pyrilamine ($M_1 = 10^{-11}$ mole/l.; $M_2 = 10^{-10}$ mole/l.; $M_3 = 10^{-9}$ mole/l.); unbroken lines, 17 ($S_1 = 10^{-8}$ mole/l.; $S_2 = 5 \times 10^{-8}$ mole/l.; $S_3 = 10^{-7}$ mole/l.; $S_4 = 10^{-6}$ mole/l.).

ethanolamine of 17, compound 29, is as active as its parent compound ($pA_2 - 14$, 29/17 = 1.0; $pA_{10} - 14$, 29/17 = 1.05) speedier in onset of action, and more easily washed out.

If a solution of compound 17 or 22 is made freshly in distilled water to which a calculated amount of NaOH is added to maintain neutrality[7] the antihistamine potency increases rapidly from zero time and is maintained unchanged for 24 hr. (see Fig. 1).

Gastric Juice.—In groups of 10 guinea pigs anesthetized with 20 mg./kg. of pentobarbital sodium intraperitoneally and protected by pretreatment with 0.1 mg./kg. of promethazine, injection of 0.1 mg. of histamine intramuscularly, repeated after 1 hr., produced a mean flow of 0.4 mmole of HCl in 5.6 ml. volume of juice in the ligated stomach. Intraperitoneal injection of 1 mg./kg. of 23 reduced the histamine-induced output of acid to 0.1 mmole and 1 mg./kg. of 30 to 0.024 mmole. This reduction is statistically significant ($p = 0.001$). In 5 cats and 5 dogs anesthetized with 33 mg./kg. of pentobarbital sodium, the flow was collected from a cannula in the pylorus during infusion of 20 μg./kg./min. of histamine over a period of 6 hr. Compounds 23 and 30 injected intravenously in a dose of 1 mg./kg. reduced or abolished the flow of acid juice. Compounds 23 and 30 had no effect on the volume or acidity of the gastric juice or the number of mucosal defects in Shay[12] rats kept narcotized with 250 mg. of urethane every 4 hr. for a 12 hr. period.

Antihistamines such as pyrilamine fail to prevent the inhibition of the rhythmical contractions of the isolated uterus of a rat in estrus. Compounds 17, 18 and 29, or 22, 23 and 30 prevent this action of histamine at concentrations in the bathing fluid of 1 μmole/l. All cause a slowing of the spontaneous rhythm but no diminution of the contraction, and in their presence the effect of added histamine is altered from inhibition to acceleration of the rhythm. Larger doses of histamine overcome this block.

Other Actions.—On intravenous injection these compounds are convulsant (LD_{50}'s range from 0.006–0.1 mole/kg.). The ethanolamines are 10–100 times less toxic. The effect is complicated by a paralysis of skeletal muscle which is decamethonium-like in type. The compounds are also local anesthetics with properties similar to procaine but weaker. They antagonize the spasmogenic action of 5-hydroxytryptamine on isolated guinea-pig ileum.

TABLE IV

VARIATIONS OF POTENCY AS ANTIEPINEPHRINE WITH VARIATIONS OF THE SUBSTITUENT ON THE RING

Substituent	Order of potency on varying the other substituent
m-H	Me > I > Br > Cl > F > Ph > H
m-Cl	Br > Cl > F > H
m-Br	Br > Cl > F > H
m-Me	Me > Br > Cl > F > H
p-H	Me > I > Br > Cl > F > H
p-F	Me > Br > Cl > H
p-Cl	Me > Br > Cl > H
p-Br	Br > Me > Cl > H
p-Me	Br > Me ≃ H

Discussion

Structural Requirements for Antiepinephrine and Antihistamine Activity

A. Substitution on the Phenolic Ring.—Antiepinephrine activity is increased by substituents on the 2-phenyl ring of DMEA (see Table IV). Antihistaminic activity is increased by disubstitution on the ring and may be increased by monosubstitution.

The relation between substitution on the 2-phenyl ring and antiepinephrine potency is summarized in Table IV. As a general rule Me ≃ I > Br > Cl > F > Ph > H. For monosubstitution *para* > *meta* for antiepinephrine activity. Substitution in both *para*- and *meta*-positions of the 2-phenyl group increase antiepinephrine and antihistamine activity. When the halogen in the *para*- or *meta*-position remains constant both antagonisms increase with the size of the other halogen substituent. The most active antiepinephrine available is the *p*-methyl *m*-bromo compound.

B. Substitution on the 2-Carbon Atom of the Ethylamine Chain.—There is an essential difference in the requirements for antiepinephrine and antihistamine activity. For the former 2-Br > 2-Cl, for the latter 2-Cl > 2-Br; for the former 2-OH is inactive, for the latter 2-OH is active. This order of potency for antiepinephrine activity in relation to halogen agrees with that found for congeners of Dibenamine; the order of potency for antihistamine activity agrees with that found in congeners of DMEA[3] but contrasts with that found in congeners of Dibenamine[6] where I ≃ Br > Cl. In contrast to the ethanolamines of other 2-halogenoalkylamines,[3,11] compounds 29 and 30 have a classical antihistamine activity equal to or a little greater than the equimolar amount of parent compound (see Table II). Dichloroisoproterenol (D.C.I.) which blocks the β-receptors for catecholamines in a surmountable manner has an antihistamine potency similar to that of the ethanolamines with two substituents on the phenyl group. Compound 29, which closely resembles D.C.I. in structure, has a weak, irregularly exerted capacity to

(12) H. Shay, S. A. Komarar, S. S. Fels, D. Meranze, M. Gruenstein and H. Siplet, *Gastroenterology*, **5**, 43 (1945).

block β-receptors which varies in different tissues and preparations (Table III). The importance of the terminal substituent on the nitrogen atom for closeness of fit to this receptor is at once apparent.

The demonstration of antagonism to the effects of histamine in inhibiting motility of rat uterus, and in causing a flow of acid gastric juice in three species reveals a new degree of antihistamine activity. The active compounds so far examined are disubstituted and Br > Cl. The activity of the ethyleneiminium picrylsulfonate relative to its parent compound 11 agrees with a previous finding for the E^+ of classical halogenoalkylamines.[11] The duration of the blockade produced by these compounds is less than that of phenoxybenzamine. If it is assumed that in the latter case an ester is formed by alkylation of the α-receptor, the hydrolysis which is necessary for regeneration may be anchimerically aided by the N,N-dimethylamino moiety in this series, which has a greater nucleophilic driving force than N,N-dialkyl groups of greater complexity. The new feature is that the antagonism is not merely brief but is surmountable; it is possible that this is due to easy reversibility of alkylating power.

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Quaternary Ammonium Compounds. III. Antiacetylcholinesterase Activity and Charge Distribution in Aromatic Quaternary Ammonium Compounds

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A series of trimethylphenylalkylammonium compounds has been prepared and examined for antiacetylcholinesterase activity. The results indicate that quaternary ammonium compounds in which the quaternized nitrogen atom is adjacent to an aromatic ring are more active than those in which the quaternary ammonium group is removed from the benzene ring and is of the aliphatic type. This phenomenon is discussed and explained in terms of charge delocalization and stereochemistry of the two types of quaternary ammonium ion.

It is generally accepted that acetylcholinesterase contains two functional entities in its active site, the esteratic site and the anionic site.[1] The substrate, usually acetylcholine, is considered to unite with the enzyme at these two points; the quaternary ammonium group at the anionic site and the acyl carbon of the ester group at the esteratic site. Reversible antiacetylcholinesterases such as eserine and neostigmine are considered to unit with the same active sites but to differ from the substrates in being hydrolyzed either not at all or only very slowly. However, many simple quaternary ammonium compounds which contain no other polarized group possess antiacetylcholinesterase activity. It is considered that the quaternary ammonium ion becomes adsorbed onto the anionic site and then the adsorbed onium ion hinders the approach of substrate molecules by one or both of two factors: (a) the electrostatic repulsion between the positively charged centers of acetylcholine and the inhibitor, and (b) the bulk of the quaternary ammonium ion prevents access to the esteratic site of the enzyme. If the substrate and inhibitor molecules are sufficiently small steric interaction at the esteratic site is not sufficient to achieve complete inhibition.[2] It follows, therefore, that there is a relationship between the forces of adsorption of quaternary ammonium compounds onto acetylcholinesterase and the antiacetylcholinesterase activity of these compounds. This has been shown to be the case by Myers.[3]

The total adsorption force between acetylcholinesterase and quaternary ammonium compounds is comprised of the following constituent forces[4,5]: (a) coulombic interaction between the positive charge of the quaternary ammonium group and the anionic site of the enzyme; (b) van der Waals forces between the hydrocarbon moiety of the quaternary ammonium ion and the enzyme suface. A third factor which is involved in the antiacetylcholinesterase activity of quaternary ammonium ions is the surface activity of amphipathic molecules or ions. As the lyophobic to lyophylic ratio is increased in an homologous series of quaternary ammonium compounds, such as *n*-alkyltrimethylammonium, so the antiacetylcholinesterase activity will increase because of (a) the increased forces of attraction between the enzyme and inhibitor, and (b) the increased concentration of onium ions at the interface of the water and enzyme due to the effect of water on the amphipathic ions. This effect will be called the "distribution effect" in the following discussion.

Thomas[6] postulated that with aliphatic quaternary ammonium ions the major contribution to the coulombic force was between the fractional positive charge on the α-carbon atoms of the quaternary ammonium group and the anionic site rather than between the positive charge on the nitrogen atom and the site. He came to this conclusion after a consideration of the charge distribution and stereochemistry of the quaternary nitrogen group and a study of the antiacetylcholinesterase activities of a series of stereospecific quaternary ammonium spiran compounds.

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