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THE ROLE OF THE CYCLIC ETHYLENEIMINIUM ION IN THE PHARMACOLOGICAL ACTIVITY OF THE 2-HALOETHYLAMINES

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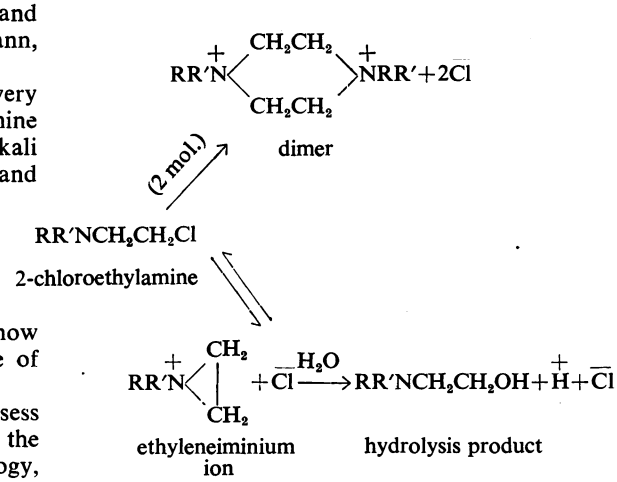
The sulphur and nitrogen "mustard" derivatives, to which the 2-halogenoethylamines are chemically related, form intermediate compounds which may react with constituents of biological systems in aqueous solution at physiological *p*H. The basic chemical reaction to which the nitrogen and sulphur "mustards" owe their specific activity is probably cyclization leading to the formation of N-substituted ethyleneiminium cations and halogen ions (Golumbic, Fruton, and Bergmann, 1946).

The ethyleneiminium ion produced is very reactive, re-forming the original haloethylamine in the presence of halogen hydracid, while alkali may convert it either to the dimer (Hanby and Rydon, 1947) or to the half-hydrolysis product. The latter can again become an ethyleneiminium ion by the cyclization of the remaining chloroethylamine group. Ethyleneiminium ions react rapidly with sodium thiosulphate and the reaction is now in general use for the estimation of this type of compound in a mixture of products.

Unlike the nitrogen "mustards," which possess two 2-chloroethylamine groups, compounds of the dibenamine type possess only one. By analogy, the latter were assumed to undergo similar changes *in vivo* to those known to be undergone by the nitrogen "mustards" *in vitro* (Nickerson, Nomaguchi, and Goodman, 1946; Nickerson and Goodman, 1948). The antiadrenaline activity was attributed to the ethyleneiminium ion, which was presumed to be formed.

Thus compounds of the dibenamine type were assumed to undergo the changes in neutral aqueous media as shown in the next column.

In the light of this hypothesis, Nickerson and Gump (1949) examined a large number of 2-haloethylamines and related compounds in an attempt to find the structural features necessary to produce antiadrenaline action in this series. Intermediate products such as dimers were prepared and found to be inactive, as also were the alcoholic end products. It was therefore assumed that the intermediate cyclic ethyleneiminium ion was the pharmacologically active species.



Chapman, James, Graham, and Lewis (1952) have shown that ethyleneiminium ions are in fact formed from haloethylamines of the dibenamine type, and Chapman *et al.* (1952) have isolated a picrylsulphonate of such an ion. 2-Fluoro-ethylamines of this type are, however, exceptional: they do not give rise to ethyleneiminium ions, whereas the corresponding chloro, bromo and iodo compounds do so readily; nor do they display the antiadrenaline and antihistamine activities so characteristic of the corresponding chloro, bromo and iodo compounds (Chapman and James, 1953; Graham and Lewis, 1953). Consequently, it

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appears that the antiadrenaline and antihistamine activities of this group of compounds are due, not to the haloethylamines themselves, but to the ethyleneiminium ions formed from them.

The purpose of this investigation was to test this assumption further and to discover whether other less specific activities were due to the unchanged 2-haloethylamines themselves.

METHODS

1. *Chemical.*—The chemical investigation was carried out by Chapman and James (Chapman, James, Graham, and Lewis, 1952). This consisted of determination of the concentration of ethyleneiminium ion, by means of sodium thiosulphate titration, in aged solutions of the four 2-haloethylamines selected, viz.: J10, J11, J12, and J13, i.e., N-α-naphthylmethyl-N-ethyl-2-haloethylamine in which the halogen was Cl, Br, I, and F respectively. A neutral aqueous-acetone solution was employed, the composition of which is given below. Curves were obtained relating the concentration of ethyleneiminium ion with age of the neutral solution. The amounts of halide ion formed in cyclization and of the hydrogen ion formed in hydrolysis of the iminium ion were also determined.

2. *Pharmacological.*—The solutions used were prepared as follows:—The compound (0.0002 moles) was dissolved in 2.5 ml. of glass-distilled water, 6 ml. of acetone added, and the solution brought to 30° C. 1 ml. of 0.2 N-sodium hydroxide was added, and the volume adjusted to 10 ml. with distilled water. This solution was maintained at a temperature of 30° C. throughout the experiment.

At various intervals of time samples were taken, diluted with saline and immediately assayed; similar solutions without the haloethylamine were used as controls. The ED99 against the pressor response to adrenaline (4 μg./kg.) in spinal cats and the ED75 against the depressor responses to histamine (2 μg./kg.) in cats anaesthetized with chloralose (60 mg./kg.) were measured. Details of the technique are given in a previous publication (Graham and Lewis, 1953).

3. *Mode of Action.*—Five pairs of cats were taken and the larger animal of each pair was injected intravenously with 20 mg./kg. of dibenamine hydrochloride. This cat (the donor) was anaesthetized with ether and chloralose (60 mg./kg.) 18–24 hours later, and the response of the blood pressure to adrenaline (4 μg./kg.) recorded. The recipient cat was similarly anaesthetized and the left carotid artery of the donor connected to the cephalic end of the right carotid artery of the recipient; the peripheral end of the femoral vein (or occasionally of the femoral artery) of the recipient was connected to the central end of the femoral vein of the donor. Cross-circulation was maintained during 1–2 hours with the aid of heparin. The cats were then separated and the response of each to injected adrenaline determined.

In these and in 10 other cats, dibenamine hydrochloride (20 mg./kg.) was injected intravenously and the response of the blood pressure to repeated injections of adrenaline (4 μg./kg.) determined after periods of 24, 48, 72, and 96 hours. Adrenaline hydrochloride in a dose of 10 mg./kg. was injected in some of these cats. In others, sodium thiosulphate (1 g./kg.) was injected over a period of 5–10 minutes, followed by continuous infusion from an automatic syringe of 0.3 g./kg./hr. Regular injections of 4 μg./kg. of adrenaline were maintained for periods up to 12 hours. In some cats both procedures were followed. Six cats were injected with J11 (10 mg./kg.) and similarly tested at varying periods up to 48 hours.

4. *Specificity. Blood Pressure.*—The responses of the blood pressure to adrenaline (4 μg./kg.), histamine (2 μg./kg.), acetylcholine (1 μg./kg.), and posterior pituitary extract (0.1 unit/kg.) were demonstrated in cats anaesthetized with chloralose (60 mg./kg.) and the effect of several doses of each haloethylamine determined by the method previously described (Graham and Lewis, 1953). The compounds used were: (*a*) the four N-α-naphthylmethyl-N-ethyl-2-haloethylamines in which the halogen was Cl (J10), Br (J11), I (J12), and F (J13); and (*b*) four N-naphthylmethyl-N-alkyl-bromoethylamines, viz., α-naphthylmethyl derivatives in which the alkyl group was ethyl (J11) or methyl (J21) and β-naphthylmethyl derivatives in which the alkyl group was ethyl (J17) or methyl (J26). An interval of one hour was allowed to elapse between injections of posterior pituitary in order to avoid tachyphylaxis.

Uterus.—Rabbit uterus was mounted in a 10-ml. modified Magnus bath in Locke's solution, oxygenated with O_2+5% CO_2 at 37° C. and *p*H 7.3. Standard responses were obtained to adrenaline (1.0–100 μg./100 ml.) and to posterior pituitary extract (0.05–0.5 unit/100 ml.), alone and in the presence of the haloethylamines (0.1 μg.–10 mg./100 ml.). The spasm-producing agent was added at intervals of 10–15 minutes and allowed to act for one minute before washing. At least four equal successive responses were obtained before a suitable dose of inhibitor was added and allowed to act for three minutes. The degree of inhibition was found for several doses of haloethylamine and the percentage inhibition plotted against the logarithm of the dose. The dose of inhibitor causing 50% inhibition was estimated from the line thus obtained.

Guinea-pig Ileum.—The spasmolytic activity of these compounds was investigated on strips of terminal guinea-pig ileum mounted in Tyrode's solution at 37° C. and *p*H 7.3. The effects of barium chloride (2.5–5.0 mg./100 ml.), acetylcholine (1.0–2.0 μg./100 ml.), and histamine (1.0–2.0 μg./100 ml.) were found alone and in the presence of atropine (0.01–500 μg./100 ml.), papaverine (0.5–5.0 mg./100 ml.), mepyramine (0.01 μg.–5.0 mg./100 ml.) and the haloethylamines (0.05 μg.–3.0 mg./100 ml.). The dose of each haloethylamine causing 50% inhibition of the spasm caused by each of the three agents was estimated and compared with that for atropine, papaverine, and mepyramine.

Results

Aged solutions of haloethylamines J10, J11, J12, and J13—viz., α-$C_{10}H_7CH_2N(Et)CH_2CH_2X$, where X=Cl (J10), Br (J11), I (J12), and F (J13), as free base, were tested by the methods 1 and 2 above.

The fluoro compound did not form any ethyleneiminium ion (Chapman, James, Graham, and Lewis, 1952). No uptake of thiosulphate or pharmacological activity was recorded. J11 and J12 cyclized very rapidly, over 98% of the theoretical maximum transformation occurring within 2 minutes. The amount of residual haloethylamine (2%) was small enough to be disregarded. With J10, the formation of the intermediate was slow, (maximum in 40 minutes) and was incomplete (maximum observed 35% of the theoretical). The residual haloethylamine was thus considerable and unless removed from solution might be active *in vivo*. Before assaying the solution of J10 the sample was washed twice with an equal volume of ether in a separating funnel immediately before injection. This additional procedure removed all uncyclized haloethylamine, leaving only the cyclic ethyleneiminium ion.

The results of the experiments are shown in Table I, where the concentrations of ethyleneiminium ions, the antiadrenaline activity and the antihistamine activity are given for solutions of various ages. In Figs. 1, 2 and 3, the curves are shown relating the percentage of ion to time, —that is, age of solution—for J10, J11, J12 respectively. The points on the three figures represent the percentage of antiadrenaline and antihistamine activity plotted against age of solution. These points fit well to the curves which represent the concentration of ethyleneiminium ion.

Table I

THE CHEMICAL REACTIVITY AND PHARMACOLOGICAL ACTIVITY OF "AGED" SOLUTIONS OF SOME HALOETHYLAMINES

Compound	Age of Solution (min.)	Antiadrenaline Activity (% of Theoretical Maximum)	Antihistamine Activity (% of Theoretical Maximum)	g. Ion Produced/Mole Reactant %
J10	5	11		6
	7·5	14	16	17
	10	20		20
	30	42	34	35
	50	42		34
	150		15	22
	180	21		19
	270		10	14
J11	0	100	100	98
	30		73	74
	60	72	60	64
	90	54		57
	180	32	40	40
	270	30		28
	360	23	20	19
	540	5		10
J12	0	100	100	98
	45	89		80
	90	70	59	66
	180	43		50
	240	38	42	40
	330	29		33
	360		29	31
	480	23		25

The course of deterioration of pharmacological activity was followed in 0.02 M solutions of J10, J11, and J12 in 3:2 (v/v) acetone-water. The table shows the percentage of theoretical maximum antiadrenaline and antihistamine activity in "aged" solutions. In the last column of the table is shown the amount of substituted ethyleneiminium ion, expressed as the percentage of the theoretical maximum. It will be noted that there is good correlation between antiadrenaline activity (column 2), antihistamine activity (column 3) and amount of ethyleneiminium ion (column 4).

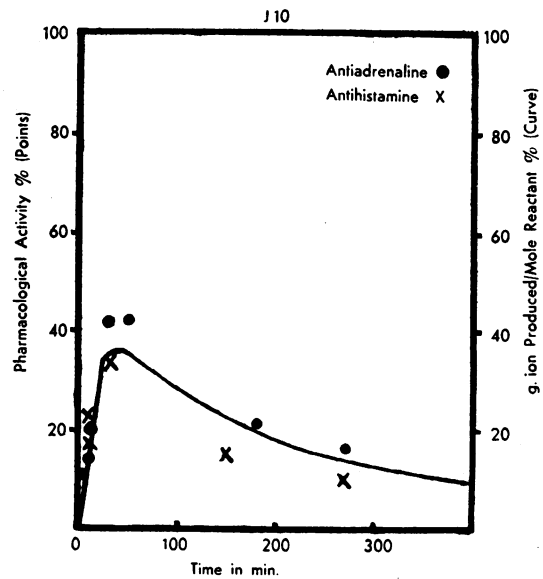

FIG. 1.—To show the relationship between pharmacological activity (antiadrenaline action and antihistamine action) and the amount of ethyleneiminium ion in an ageing solution containing 0.02 M. J10 in 3:2, v/v, acetone-water. The curve indicates the amount of ethyleneiminium ion present expressed as a percentage of the theoretical maximum of g. ion produced per mole of J10. The antiadrenaline activity was assayed against the pressor responses to adrenaline in spinal cats, and the antihistamine activity against the depressor response to histamine in cats anaesthetized with chloralose.

The activity possessed by freshly prepared aqueous-acetone solutions of J11 or J12 was designated as 100%. This could not be done with J10, so solutions were assayed 40 minutes after they had been made—i.e., when the concentration of ethyleneiminium ion was chemically estimated to be 35% of the theoretical maximum; the results were adjusted accordingly.

It must be emphasized that the chemical estimations were made on solutions very different from blood or other body fluids. It cannot be assumed that the reactions: haloethylamine→ethyleneiminium ion→hydrolysis product, will follow the same time course when a haloethylamine is injected into an animal as they do in artificial systems; but the ageing solution provides samples

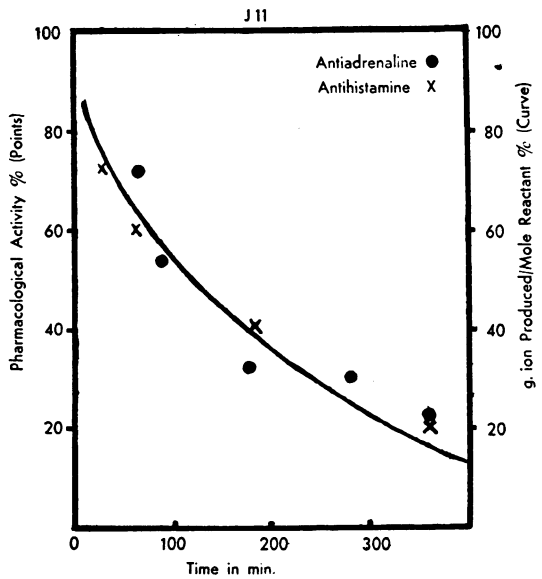

FIG. 2.—To show the relationship between pharmacological activity (antiadrenaline action and antihistamine action) and the amount of ethyleneiminium ion in an ageing solution containing 0.02 M. J11 in 3: 2, v/v, acetone-water. As in Fig. 1, the curve represents percentage of ion and the points represent percentage of pharmacological activity. With this compound, as with J10, both sets of points fit the curve.

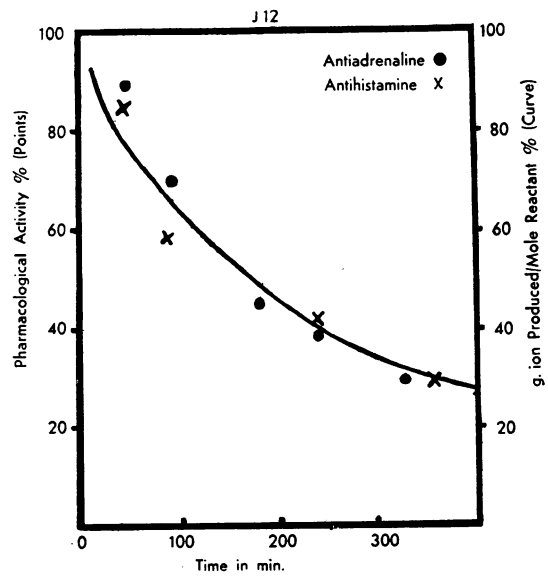

FIG. 3.—To show the results of experiments similar to those illustrated in Figs. 1 and 2, using J12. Again both sets of points fit the curve.

of the ethyleneiminium ion of different, but known, concentrations, and the potencies of these samples were estimated biologically, so that the potency could be correlated with the ethyleneiminium ion content. Thus in Fig. 2, when the solution was one hour old, it contained 64% of the compound J11 present as ethyleneiminium ion; at this time the solution had 72% of its original antiadrenaline activity and 60% of its original antihistamine activity. After three hours, the solution contained 40% of ethyleneiminium ion, 32% of its antiadrenaline activity and 40% of its antihistamine activity. Similarly, the other points lie on the curve, as also do the points in Figs. 1 and 3, for J10 and J12 respectively. The antiadrenaline and antihistamine activities are thus directly proportional to the amount of ethyleneiminium ion present in solution.

The results of the experiments on the responses of cat blood pressure to adrenaline, histamine, acetylcholine and posterior pituitary extract are shown in Table II. The pressor response to adrenaline was reversed by all the compounds in the table except J13 (a fluoro derivative). The depressor response to histamine was reduced by all the compounds, but J13 only caused slight reduction in the response after a very large dose. The pressor response to posterior pituitary extract was not reduced by any of the compounds, and the depressor responses to acetylcholine were only slightly diminished by some of the haloethylamines.

TABLE II

THE ACTION OF THE HALOETHYLAMINES ON THE RESPONSE OF BLOOD PRESSURE TO ADRENALINE, HISTAMINE, ACETYLCHOLINE AND PITUITARY (POSTERIOR LOBE) EXTRACT. (MEAN OF 3 READINGS ON 3 ANIMALS)

Compound	Dose (mg./kg.)	Lowering of B.P. (% of Initial)	% Reduction in Responses of B.P. after Admin. of Haloethylamines			
			Adrenaline (4 μg./kg.)	Histamine (2 μg./kg.)	Acetylcholine (1 μg./kg.)	Post. Pit. (0·1 unit kg.)
J10	0·05	0	5	24	3	5
	0·5	10	R	85	8	0
J11	0·05	3	10	35	10	0
	0·5	9	R	84	16	0
J12	0·05	0	18	40	0	0
	0·5	0	R	74	0	0
J13	0·05	0	P	0	4	0
	0·5	0	P	4	0	0
	10·0	0	P	37	0	0
J17	0·05	7	10	2	0	0
	0·5	12	R	62	0	0
J21	0·05	2	P	2	0	0
	0·5	8	45	27	4	0
	1·0	14	R	43	9	0
J26	0·05	0	P	10	2	0
	0·5	0	5	20	0	0
	3·0	10	R	50	0	0

P = Potentiation of pressor response to adrenaline 12–200%.
R = Reversal of pressor response to adrenaline.

The table shows the effect of the haloethylamines on the responses of cat blood pressure (anaesthetic; chloralose 60 mg./kg.) to adrenaline 4 μg./kg., histamine 2 μg./kg., acetylcholine 1 μg./kg., and pituitary (posterior lobe) extract 0.1 unit/kg. All the active compounds show great specificity in inhibiting responses to adrenaline and histamine.

In the cross-circulation experiments it was found that 18–24 hours after injection of 20 mg./kg. of dibenamine the response of the donor cat to 4 μg./kg. of adrenaline was reversed. There was insufficient dibenamine in the circulation of the donor to effect an antagonism to adrenaline in the recipient cat. In the donor cats and others similarly injected with dibenamine or J11 the "blockade was absolute," a reversal of the usual pressor response to adrenaline persisting with doses of 10 mg./kg. Only towards the end of the fourth day (dibenamine) or the second day (J11) did a competitive phase precede the disappearance of the effect of the antagonist.

At no time (2 hours to 4 days after dibenamine, 1–24 hours after J11) did injection or infusion, or both, of thiosulphate modify the effect or duration of action of dibenamine or J11.

On every such occasion tested, the effect of massive doses of adrenaline (1–10 mg./kg.) was to produce a profound fall in blood pressure. After the pressure had returned to its previous level, further injections of 4 μg./kg. produced either no response or a small pressor response associated with cardiac stimulation. This phase lasted about 30 minutes, was followed by renewal of the reversal, and the cycle could be repeated several times by injection of the large dose of adrenaline. Injection or infusion of thiosulphate after the adrenaline did not modify this phenomenon, which was probably due to temporary exhaustion of the depressor mechanism by large doses of adrenaline.

The spasmolytic action of the haloethylamines was examined on guinea-pig ileum. The results are presented in Table III, where the potencies are expressed as the dose causing a 50% reduction in the response to spasmogenic agents. Comparisons are made with atropine for antiacetylcholine action, with mepyramine for antihistamine action and with papaverine. It can be seen from Table III that the haloethylamines are essentially specific antihistamines, whilst exerting some antagonism of the responses to acetylcholine.

TABLE III

THE ACTION OF THE HALOETHYLAMINES ON THE RESPONSES OF GUINEA-PIG ILEUM TO HISTAMINE, ACETYLCHOLINE AND BARIUM CHLORIDE

Compound	Dose of Compound in μg./100 ml. Causing 50% Inhibition of Response to:		
	$BaCl_2$ (2·5–5 mg./100 ml.)	Acetylcholine (1–2 μg./100 ml.)	Histamine (1–2 μg./100 ml.)
Atropine ..	120	0·1	100
Papaverine ..	2,100	2,300	2,000
Mepyramine ..	1,250	2,000	0·14
J10	>2,000	100	0·19
J11	>2,000	75	0·35
J12	>2,000	150	0·33
J13	>2,000	>5,000	700
J17	>2,000	300	0·4
J21	>2,000	200	10·0
J26	>2,000	300	15·0

Guinea-pig ileum mounted in a modified Magnus bath in Tyrode's solution, at 37° C., pH 7.3, oxygenated with O_2 + 5% CO_2. The table shows the dose of compound in μg./100 ml. causing 50% inhibition of the responses to barium chloride 2.5–5 mg./100 ml., acetylcholine 1–2 μg./100 ml., and histamine 1–2 μg./100 ml.

TABLE IV

THE EFFECT OF THE HALOETHYLAMINES ON THE CONTRACTIONS OF ISOLATED RABBIT UTERUS TO ADRENALINE AND PITUITARY (POSTERIOR LOBE) EXTRACT

Compound	Dose of Compound Producing 50% Inhibition of Response to:	
	Adrenaline (1–100 μg./100 ml.)	Post. Pituitary (0·05–0·5 unit/100 ml.)
J10	0·8	7,000
J11	0·7	5,000
J12	1·5	10,000
J13	700	7,000
J17	1·6	5,000
J21	30·0	6,000
J26	50·0	10,000

Isolated rabbit uterus mounted in Locke's solution in a modified Magnus bath at pH 7.3, and 37° C. oxygenated with 5% CO_2 in O_2.

Standard additions of stimulating agents were made at 10–15 min. intervals and repeated after addition of a haloethylamine. The values for 50% inhibition were obtained from a plot of percentage inhibition against the logarithm of dose of the haloethylamine.

The specificity of the antagonism of the responses to adrenaline is clearly shown.

The results of the experiments on rabbit uterus are given in Table IV. Here again the haloethylamines are shown to be specific—this time as anti-adrenaline agents.

DISCUSSION

A quantitative relationship between the concentration of the ethyleneiminium ion formed by J10, J11, and J12 and the specific pharmacological activity of these compounds has been observed. As the haloethylamines are known to form the ethyleneiminium ion *in vitro*, and the pharmacological activity of the haloethylamines is known to vary directly with the concentration of ethyleneiminium ion, it may be deduced that such cyclic ions are formed when the haloethylamines are injected *in vivo* and that they are the active species. Thus the correctness of Nickerson's supposition, that the pharmacological activity of these compounds is due to the formation of active intermediates, has been rendered more probable by experimental evidence.

A serious objection to this view has been raised by Axelrod, Aronow and Brodie (1952), who examined the distribution and metabolic fate of dibenamine in the dog. According to the present theory, dibenamine would be cyclized and sub-

sequently converted to dibenamine alcohol (N-dibenzylaminoethanol). These authors were unable to detect the latter substance in the urine after administration of dibenamine. In order to determine whether or not the alcohol itself was metabolized, they injected dibenamine alcohol 30 mg./kg. into two dogs and found the proportion excreted in the urine to be 10–15%. They concluded from this that dibenamine alcohol was *not* metabolized, and used the conclusion as evidence that the alcohol was not formed from dibenamine in the body, suggesting that the antiadrenaline action was not due to the ethyleneiminium ion. It seems reasonable to ask, however, how the 85–90% of the alcohol was lost if not as a result of metabolic activity.

Axelrod *et al.* further showed that five minutes after injection of dibenamine, 60% of the latter is lost (see Table 5 of their paper). If this were converted to the corresponding alcohol, then 90% of the latter would " disappear " leaving approximately 6% of the original dibenamine to be present in the urine as alcohol. As the stated error for the method of estimation is ±5%, the argument against this mechanism occurring appears to be weak. If the 60% of dibenamine was not immediately metabolized but was held in combination by cell constituents, it would be expected that a slow release of the amine from combination would give rise to a slow formation of alcohol which would consequently be metabolized before reaching the urine.

If it be accepted that the presence of the ethyleneiminium ion is essential for the specific effect of the haloethylamines in antagonizing the actions of adrenaline and histamine, it is possible to consider the actions of these compounds in more detail. It has been shown that the compounds vary in their antiadrenaline and antihistamine potency. If the activity depends upon the ethyleneiminium ion formed, it would be logical to conclude that the structural features present in J21 and J26 as compared with those of J11 and J17 reduce the ability of the two former compounds to form the cyclic intermediate.

These compounds at higher concentrations reduce the response of guinea-pig ileum to acetylcholine. Their potencies in this respect do not bear the same relationship to one another as do their antihistamine potencies. The fact that a very high concentration of the fluoroethylamine J13 is needed indicates that activity resides in the ion rather than in the molecule. The concentrations required to reduce the responses of guinea-pig ileum to barium chloride or of rabbit uterus to pituitary extract are such as to preclude any accurate measurement.

We have observed previously (Graham and Lewis, 1953) that the group of haloethylamines examined exhibited two types of toxic symptoms, stimulation and depression. The former was quick in onset and caused death within 1 to 2 hours, whereas the depression was slower in onset and death occurred 12 to 48 hours after administration. The fluoro derivatives which do not form the cyclic ethyleneiminium ion caused a rapid convulsive death ; the chloroethylamines which cyclize less readily than the bromo or iodo derivatives (see Fig. 1) caused some initial stimulation followed by prolonged depression ; while the bromo- and iodoethylamines which rapidly and completely cyclize gave rise to a prolonged depression with delayed death. It would appear that the original haloethylamine molecule causes convulsive symptoms leading to a rapid death, while the cyclized ion formed from it causes a state of prostration in which the onset of death is delayed.

Mode of Action

The facts already discovered about the haloethylamines (Nickerson and Nomaguchi, 1948 ; Graham and Lewis, 1953) suggest that they act by blocking the receptor mechanism in certain smooth muscle cells which respond to adrenaline with a motor response (Ahlquist, 1948), but not in others which relax, or in the heart.

Nickerson and Goodman (1948) suggested that dibenamine reacted irreversibly with some cellular constituent which is essential for reaction to adrenaline stimulation. They reached this conclusion from the indirect evidence that the action of the drug may persist for several days beyond the period that an effective concentration can be demonstrated in the body. The test consisted of cross-circulation in dogs, the first dog having received a large dose of dibenamine. The time was noted when cross-circulation failed to produce antiadrenaline action in a second dog. Our experiments confirm these results in cats.

Axelrod *et al.* showed that some 20% of dibenamine was localized in fat two hours after injection into a dog, and that formation of dibenzylamine was also important (24% unchanged dibenamine recovered from all tissues of a dog five minutes after injection, and another 16% as dibenzylamine ; this leaves 60% unaccounted for). The prolonged action of dibenamine is attributed by these authors to gradual release from the fat depôt, so that the drug is present though chemically undetectable in blood the whole of the time

that its action is evident. After injection of 47 mg./kg. in a dog the amount of dibenamine absorbed in the fat was estimated to be 26 mg./kg. at two hours and 24 mg./kg. at 27 hours.

We have carried out a series of experiments in order to test whether the prolonged action of dibenamine was due to the release of the compound from fat depôts. It has been shown that administration of thiosulphate before injection of the anti-adrenaline compound prevents the antagonism of the pressor response to adrenaline from developing. The mechanism is by chemical interaction between the thiosulphate and the antagonist. It has also been observed that administration of thiosulphate after the antagonism has been established does not affect its course. We have extended these studies, having administered sodium thiosulphate by injection and by continuous infusion into cats injected intravenously with 20 mg./kg. of dibenamine 18–24 hours previously. After this time the dibenamine in the circulation is not detectable biologically by cross-circulation, so that the prolonged action is due either to release from fat depôts (Axelrod *et al.*) or to a stable dibenamine-receptor complex (Nickerson). After treatment with thiosulphate any dibenamine in the circulation would be neutralized so that release from fat depôts would be ineffective. In such circumstances, the duration of dibenamine antagonism would be brief. This is not so (up to 12 hours). Nevertheless a relationship exists between the dose of antagonist administered and the duration of the antagonism; thus, with J11, 1 mg./kg. antagonizes adrenaline for 6–8 hours, 10 mg./kg. for approximately 48 hours; with dibenamine, 5 mg./kg. lasts for approximately 36 hours, 20 mg./kg. for four days. Thus the absorption of dibenamine into the fat, although it may play some part, is not the limiting factor in the prolonged action of the drug.

Further proof of a chemical reaction with tissue constituents has been afforded by the observations of Ferguson and Wescoe (1950), who examined the action of N-dimethyl-2-chloro-2-phenylethylamine ($C_6H_5CHClCH_2NMe_2$) and its intermediate products. Two important observations were made, namely, that the duration of the antiadrenaline action *in vivo* was greater than the time in which the ion is hydrolysed to the inactive alcohol in serum *in vitro*, and that thiosulphate, which reacts with the ethyleneiminium ion and prevents its pharmacological activity, does not affect the degree of inhibition once established.

We have also obtained indirect evidence in support of this hypothesis in that the action of the haloethylamines *in vitro* is not removed by washing, while that *in vivo*, although durable, is reversible. Thus, a reaction, with some essential constituent that can eventually be replenished *in vivo* and cannot be reversed by washing a tissue *in vitro*, is indicated.

Graham and Lewis (1951, 1953) have compared the antihistamine and antiadrenaline actions of the haloalkylamines. While these activities have many features in common there are certain differences. Both the antagonisms are exerted within a very short time of administration *in vivo*, but whereas that to adrenaline continues for many hours the antihistamine action is of comparatively short duration.

In very small doses the haloethylamines potentiate the pressor response to adrenaline, probably owing to the known inhibition of amine oxidase; but there is no potentiation of histamine, nor is there any effect on histaminase. The action is due to purely structural factors, as the fluoroethylamines which do not form active intermediates cause inhibition of monoamine oxidase and markedly potentiate the pressor response to adrenaline (see Table II). In slightly larger doses active haloethylamines exert a competitive inhibition of the responses to adrenaline. This phase may be explained by assuming that below the limiting concentration for non-competitive antagonism, some of the receptors would not be occupied by antagonist and would thus be still available to adrenaline. Alternatively, there is a limiting concentration of the antagonist below which it will not attach itself to the receptor sufficiently strongly to overcome the active factors which remove and destroy it. In higher doses the pressor response to adrenaline is inhibited in a non-competitive manner, while the depressor response to histamine is always inhibited competitively at all dose levels, —again indicating a difference in the nature of the interaction of the antagonist and the two types of receptor.

Summary

1. The examination of a group of haloethylamines reported in a previous publication has been extended to a study of their mechanism of action.

2. It is more than probable that the antiadrenaline and antihistamine actions of the haloethylamines are due to the formation of cyclic ethyleneiminium ions.

3. The actions of the compounds have been explained in the light of these findings. The specific antiadrenaline and antihistamine actions,

as well as a weak antiacetylcholine action, are exerted by the cyclized ion, the non-specific actions by the molecule.

4. Their mode of action as antiadrenaline agents is possibly chemical combination with the tissue receptors, but as antihistamines they may act by forming a less stable complex with some constituent of the effector mechanism.

5. No evidence was obtained in support of the view that continuing release of haloethylamines from fat depôts is the limiting factor in the prolonged action of these compounds.

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