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POTENTIATION OF PHARMACOLOGICAL EFFECTS OF HISTAMINE BY HISTAMINASE INHIBITORS

By O. ARUNLAKSHANA*, J. L. MONGAR AND H. O. SCHILD

From the Department of Pharmacology, University College, London

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Best showed in 1929 that extracts of animal tissues destroy histamine and named the enzyme system concerned in this reaction histaminase. In 1938 Zeller described an enzyme, diamine oxidase, which destroys the diamines putrescine and cadaverine (Zeller, 1938*a*), and subsequently concluded that histaminase and diamine oxidase were identical (Zeller, 1938*b*). This conclusion was based on the finding of a strict parallelism between destruction of histamine and cadaverine by diamine oxidase preparations from different sources; competitive inhibition of diamine oxidase by histamine and cadaverine; and inhibition of the destruction of both substances by antagonists of diamine oxidase. More recently, however, Kapeller-Adler (1949, 1951) has questioned the identity of the two enzymes.

All carbonyl reagents so far examined inhibit diamine oxidase, often in very low concentrations (Zeller, 1942). Zeller has shown that semicarbazide, thiosemicarbazide and hydroxylamine produce this effect, and has suggested that diamine oxidase itself contains carbonyl groups which interact with diamines and are blocked by carbonyl reagents. Various other compounds also inhibit diamine oxidase, though less actively, e.g. guanidine and iminazole and their derivatives. One of the derivatives of guanidine, however, aminoguanidine, is a powerful inhibitor of diamine oxidase (Schuler, 1952), and it is probably relevant that this compound possesses a reactive amino group like the typical carbonyl reagents.

The present experiments started with the observation that in the presence of semicarbazide the action of histamine on guinea-pig intestine was markedly potentiated. Then it was found that other effects of histamine were also potentiated and that other histaminase inhibitors acted like semicarbazide. Experiments dealing with the potentiation of histamine by histaminase inhibitors are described in the first part of this paper. The experiments described in the second part were designed to test whether the potentiating effects of

* British Council Scholar.

histaminase inhibitors are a direct consequence of their action on the enzyme. It was found that the tissues in which potentiation occurred contained appreciable amounts of histaminase. A quantitative comparison was made of the histaminase inhibiting and potentiating activity of various types of compounds, and the effect of several of the compounds was tested on certain analogues of histamine which, though pharmacologically closely related to histamine, are resistant to histaminase. A preliminary account of some of this work has been published (Mongar & Schild, 1951).

METHODS

The isolated ileum, tracheal chain and uterus of young guinea-pigs were used. The tracheal chain was prepared by tying rings of trachea in such a manner that the muscular portions were aligned. The ileum was suspended in a 1 ml. bath in Tyrode solution (NaCl 8·0, KCl 0·2, $CaCl_2$ anhyd. 0·2, $MgCl_2$ 0·1, $NaHCO_3$ 1·0, NaH_2PO_4 0·05, glucose 1·0 g/l.) stirred with O_2; the uterus in a 5 ml. bath in Tyrode solution and the tracheal chain in a 5 ml. bath in Krebs's solution (NaCl 6·9, KCl 0·43, $CaCl_2$ 0·28, $MgSO_4$ 0·14, NaH_2PO_4 0·14, $NaHCO_3$ 2·1, glucose 1·0 g/l.) stirred with $O_2 + 5\%$ CO_2. All experiments on isolated tissues were carried out at 37° C using automatic assay apparatus. The isolated organ bath and accessories are shown in Fig. 1. The automatic switching unit is described in detail elsewhere (Boura, Mongar & Schild, 1954). It is a development of the principle already described (Schild, 1947*a*). By means of this apparatus the various operations of filling and emptying the bath are carried out mechanically. In the experiments using the ileum the drugs were also administered automatically by completely replacing the bath solution with pre-warmed solution of the drug. The experiments with uterus and tracheal chain were done using the automatic apparatus, but in this case the drugs were injected by hand. The chief advantage of the automatic method is that the preparations respond very uniformly and can be stabilized by a preliminary series of doses of histamine before introducing the potentiating drug. In this way a small potentiation such as is frequently produced by histaminase inhibitors can be detected with a degree of confidence not otherwise attainable.

The usual procedure was first to establish a concentration–action curve for histamine, then to stabilize the preparation by constant doses of histamine and finally to add the potentiating drug whilst continuing to administer histamine at regular intervals. These intervals were as a rule 1 min for the ileum, 3–5 min for the uterus and 30 min for the tracheal chain. The potentiating drug was added either to the washing solution alone or to both washing and drug solutions.

Inhibition of histaminase. Histaminase was used in the form of a crude acetone-dried powder prepared from pig kidney cortex. Two ml. of 2×10^{-7} histamine solution in phosphate buffer of pH 7·2 were incubated with 20 mg of the dry enzyme powder and varying concentrations of inhibitor in stoppered flasks in an atmosphere of oxygen. The flasks were shaken in a bath at 37° C for 30 min. The histamine not destroyed was determined biologically after heat-inactivation of the histaminase. Careful four-point assays were necessary to obtain a quantitative measure of the destruction.

Measurement of histaminase activity by a colorimetric method. In a limited number of experiments involving high substrate concentrations (10^{-2} M), destruction of the substrate (histamine or its analogues) was estimated chemically by determining the ammonia produced. 0·5 ml. of the heat-inactivated solution was placed in the outer cup of a Conway micro-diffusion unit along with 0·7 ml. saturated potassium carbonate solution. The inner cup contained 1 ml. of N/100 sulphuric acid. One hour at room temperature was allowed for equilibration. Then 0·4 ml. of the contents of the inner cup were pipetted into a 1 cm micro-absorption cell of the Spekker colorimeter, 0·05 ml. of freshly filtered Nessler reagent were added and the solutions were thoroughly mixed. Absorption was measured immediately using Spectrum no. 2 (blue) filters. A blank was obtained by incubating enzyme with buffer solution in the absence of substrate.

Histaminase content of isolated guinea-pig tissues. The tissues were cut into fragments weighing about 5 mg and incubated, at 37° C with shaking, in 10^{-7} histamine solution (10 ml./100 mg tissue). The histamine not destroyed by the enzyme in the tissue was determined at half-hourly intervals by withdrawing small samples for biological assay.

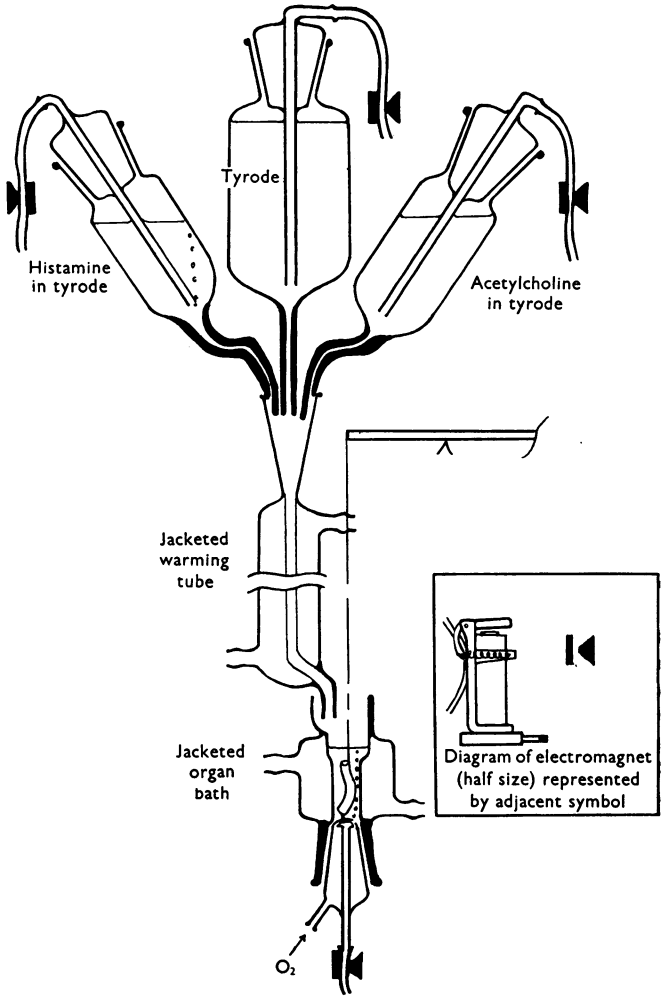


Fig. 1. Diagram of isolated organ bath and accessories used in potentiation experiments on the guinea-pig ileum preparation. The particular arrangement shown was used for giving alternate doses of histamine and acetylcholine. Immediately after emptying out the washing solution an amount of drug solution just sufficient to fill the bath flows in via the jacketed warming tube. On completion of the contraction the bath is again emptied and Tyrode washing solution flows in. These operations are carried out by electro-magnets (shown in detail in insert) which compress rubber tubing; their timing is automatically controlled. After giving repeated doses of stimulant drug to establish a steady preparation a potentiating drug is added to the solutions.

The following drugs were used: histamine acid phosphate (B.D.H.); acetylcholine chloride (Roche); semicarbazide hydrochloride (B.D.H.); B_1 pyrimidine hydrochloride (2-methyl-4-amino-5-methylamino pyrimidine) (Roche); hydroxylamine hydrochloride (B.D.H.); cadaverine (pentamethylene diamine dihydrochloride) (Light and Co.); guanidine hydrochloride, methyl guanidine sulphate and *asym.*dimethyl guanidine sulphate (B.D.H.); amino-guanidine bicarbonate (Light); iminazole (Roche); dimethyl iminazole (B.D.H.); choline chloride (B.D.H.); physostigmine salicylate (B.D.H.); β-2-pyridylethylamine hydrochloride (Maltbie Chemical Co., Newark, N.J.); β-3-pyrazolethylamine hydrochloride (Eli Lilly Chemical Co.). The concentration of all compounds is based on these salts except for histamine where it refers to the free base. All concentrations are expressed in w/v unless molar concentrations are explicitly mentioned.

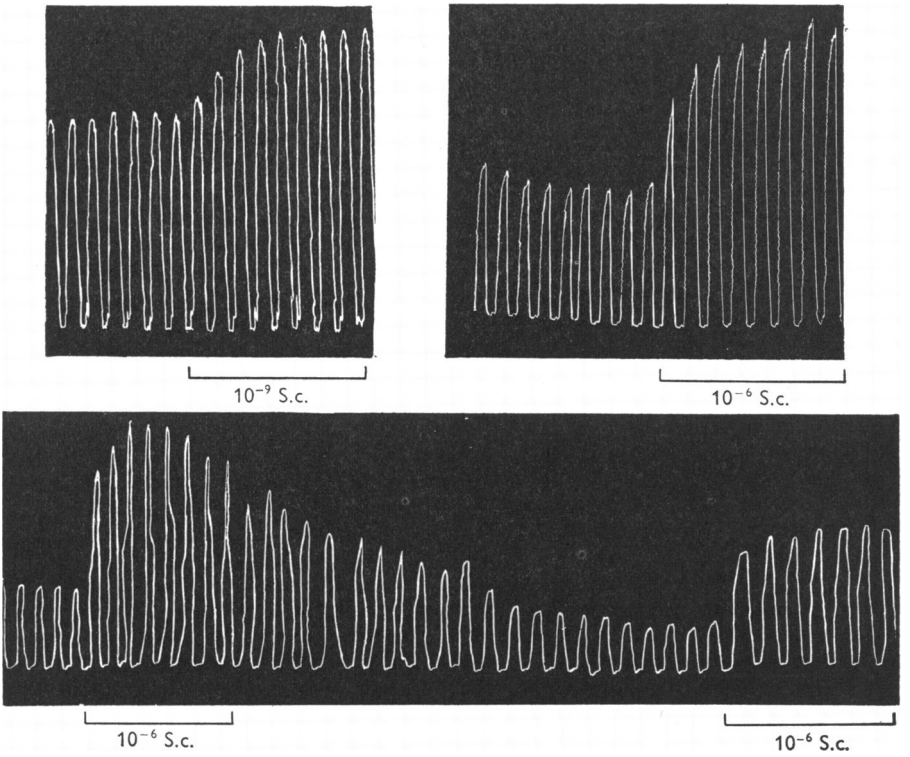


Fig. 2. Potentiations using guinea-pig ileum preparation in Tyrode. Contractions produced by 5×10^{-9} histamine. Semicarbazide (s.c.) was added to the solutions for the periods indicated. An appreciable potentiation is produced by a concentration of 10^{-9}; 10^{-6} gives a greater effect. The lower tracing illustrates reversibility of potentiation.

RESULTS

Part I. Potentiation of histamine

Guinea-pig ileum

Semicarbazide. The effect of semicarbazide on the contractions produced by histamine in the guinea-pig ileum is shown in Fig. 2. The addition of 10^{-9} semicarbazide to the Tyrode washing solution caused slight potentiation, and

10^{-6} marked potentiation corresponding approximately to a doubling of the dose of histamine. Semicarbazide itself produced no contraction of the ileum as shown by the fact that the base-line remained unchanged even though semicarbazide had been added to the washing solution. The potentiating effect increased during the course of several histamine contractions and then remained more or less constant. When semicarbazide was omitted, the potentiation was reversed. This cycle of operations could usually be repeated several times.

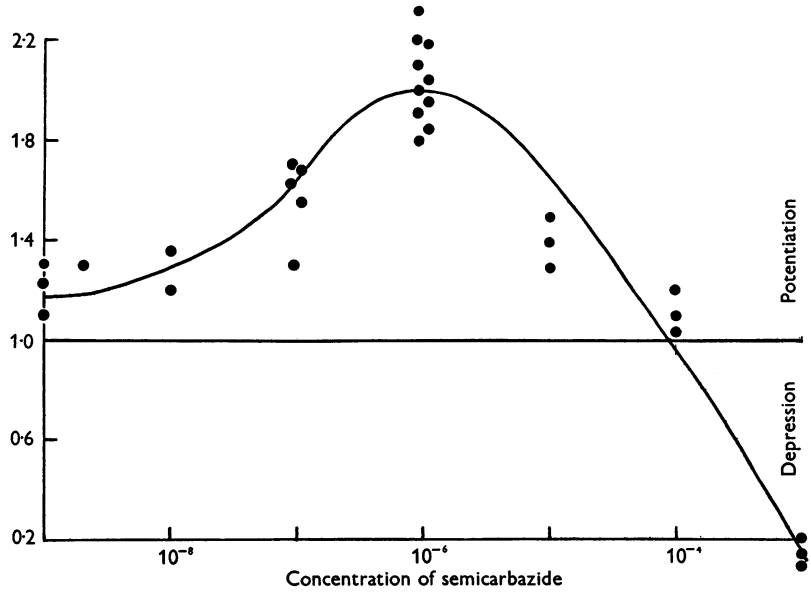


Fig. 3. Effect of concentration of semicarbazide on potentiation of histamine contractions of guinea-pig ileum. The potentiation (ordinate) is expressed in terms of the dose of histamine that would be required in the absence of potentiator to produce the same effect. Each point represents an experiment on a fresh preparation. The degree of potentiation depends on the concentration of semicarbazide used. High concentrations of potentiator depress the histamine responses.

The most consistent potentiations are obtained in fresh preparations which have been stabilized by repeated frequent doses of histamine added at intervals of 60–80 sec, and which show a steep dose-response curve with histamine itself. Since potentiation and histamine 'slope' are apparently correlated, the degree of potentiation has as a rule been expressed in terms of the equivalent dose of histamine rather than in terms of the effect produced. The degree of potentiation depends on the concentration of semicarbazide. This is shown in Fig. 3 which summarizes the results of a series of experiments extending over several months. Maximum potentiation occurs with 10^{-6} semicarbazide, higher concentrations are less effective, and concentrations above 10^{-4} depress the

response to histamine. There is considerable variation in the degree of potentiation produced by any one concentration of semicarbazide in different preparations. At best the degree of potentiation of histamine by histaminase inhibitors in the guinea-pig ileum preparation is much less than the corresponding potentiation of acetylcholine by eserine (cf. Fig. 13*b*).

Other antihistaminases

A number of other known inhibitors of diamine oxidase or histaminase were similarly tested, and all were found to potentiate the effects of histamine on guinea-pig ileum. These substances can be divided into four classes: typical carbonyl reagents, diamines, guanidine and derivatives, and glyoxaline and derivatives. A summary of results is given in Table 1.

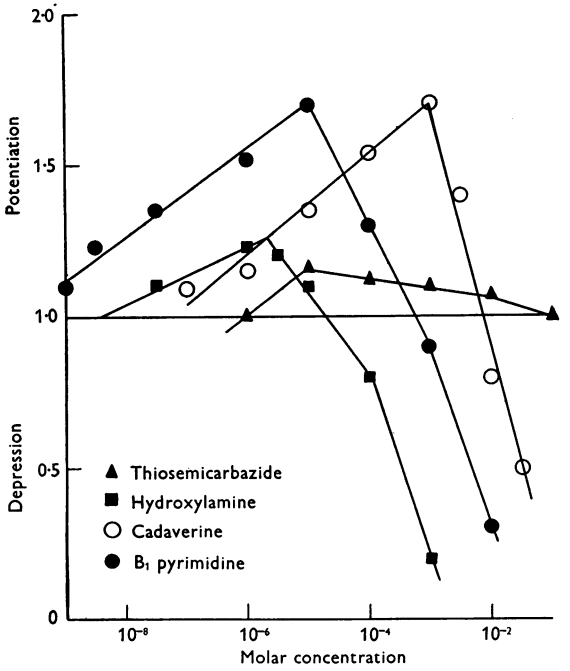
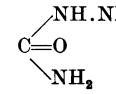
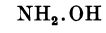
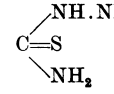
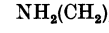
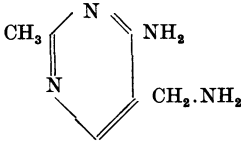
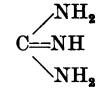
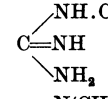
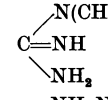
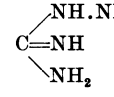
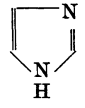
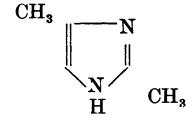


Fig. 4. Effect of concentration of carbonyl reagents and diamines on potentiation of histamine contractions of guinea-pig ileum. Each point represents an average of three or four experiments. Low concentrations potentiate, high concentrations depress.

Carbonyl reagents. Besides semicarbazide (I) two other typical carbonyl reagents were investigated, hydroxylamine (II) and thiosemicarbazide (III). These compounds potentiate histamine in low concentrations; they tend to depress in high concentrations (Fig. 4), but they are less active than semicarbazide both with regard to threshold dose and degree of potentiation.

Table 1 shows that the mean potentiation at the optimum concentration was only 1·23 for hydroxylamine and 1·15 for thiosemicarbazide. Hydroxylamine

TABLE 1. Summary of potentiation results with eleven compounds tested on the guinea-pig ileum. Potentiations are expressed in terms of the dose of histamine that would be required in the absence of potentiator to produce the same effect.

Compound	No.	Formula	Optimum concn. (M)	Mean potentiation	No. of results at this concn.
Semicarbazide	I		10^{-5}	1·95	11
Hydroxylamine	II		10^{-6}	1·23	3
Thiosemicarbazide	III		10^{-5}	1·15	5
Cadaverine	IV		10^{-3}	1·7	2
B_1 pyrimidine	V		10^{-5}	1·7	4
Guanidine	VI		10^{-2}*	1·4	1
Methyl-guanidine	VII		10^{-3}*	1·8	1
Dimethyl-guanidine	VIII		$2{\cdot}5 \times 10^{-4}$*	2·0	1
Amino-guanidine	IX		10^{-6}	1·7	3
Iminazole	X		10^{-3}*	2·0	4
Dimethyl-iminazole	XI		10^{-4}*	1·8	2

* In higher concentrations these compounds produce a rise of base-line.

has a strong depressor component, and it is possible that this may mask, to some extent, its potentiating effect.

Diamines. The diamines cadaverine (IV) and B_1 pyrimidine (V) (2-methyl-4-amino-5-methylamino pyrimidine) were tested. Zeller, Schär & Staehlin (1939) found that histamine and cadaverine acted as competitive inhibitors of diamine oxidase and that the affinity of diamine oxidase for cadaverine was of the same order as for histamine. The use of B_1 pyrimidine (an intermediate in the synthesis of vitamin B_1) was suggested by Prof. F. Bergel. The compound has not previously been found to inhibit histaminase but, as will be shown in a later section of this paper, it has a marked antihistaminase activity.

Both diamines produce potentiation, cadaverine producing maximum potentiation at a concentration of approximately 10^{-3} M (Fig. 4). B_1 pyrimidine, one of the most active potentiators encountered, is 100 times more active than cadaverine. It has been used, along with semicarbazide, to test the effect of histaminase inhibitors in tissues other than the ileum.

Guanidine derivatives. Guanidine (VI) and dimethylguanidine (VIII) were found by Blaschko (1939) to be competitive inhibitors of histaminase. Both compounds, as well as methylguanidine (VII), potentiate histamine on guinea-pig ileum, but their effects differ in several respects from those of semicarbazide. The guanidine derivatives potentiate only at relatively high concentrations, and their concentration–action curves exhibit no maximum but a graded increase of effect with dose (Fig. 5). In contrast to the carbonyl reagents, very high doses of the guanidines have a stimulant action of their own on plain muscle. They tend to produce a permanent rise of base-line when they are added to the Tyrode solution, and under these conditions it is difficult to measure the degree of potentiation quantitatively. In Fig. 5 those potentiation effects which were accompanied by a rise of base-line are shown by dotted lines.

Although derived from guanidine, aminoguanidine (IX) can be classed as a carbonyl reagent differing from semicarbazide only by the substitution of the =NH group for =O on the central carbon atom. Schuler (1952) investigated the effect of aminoguanidine on diamine oxidase from hog kidney using cadaverine as substrate, and concluded that it was the most active inhibitor of diamine oxidase known at present. Aminoguanidine became available to us only after completion of this series of experiments. It could be shown that it strongly potentiated histamine on guinea-pig ileum in a concentration of 10^{-6} M.

Iminazoles. Iminazole (X) (glyoxaline) has been shown to inhibit histaminase (Gebauer-Fuelnegg & Alt, 1932) and diamine oxidase (Zeller, 1941). Morris & Dragstedt (1945) found that large concentrations of iminazole (1500 times the concentration of histamine) antagonized the effect of histamine on guinea-pig ileum. In our own experiments concentrations from 10^{-6} to 10^{-3} M iminazole consistently potentiated the effect of histamine on the guinea-pig ileum (Fig. 5).

Above 10^{-3} M this compound had a direct stimulating action of its own. Dimethyliminazole (XI) had a similar though stronger potentiating effect than iminazole.

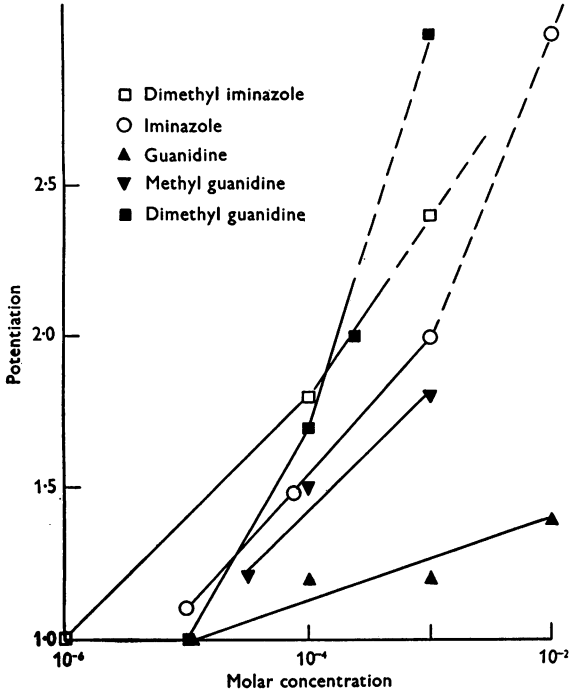


Fig. 5. Effect of concentration of guanidines and iminazoles on potentiation of histamine contractions of guinea-pig ileum. High concentrations tend to have a direct stimulant action of their own. Experiments in which a rise of base-line occurred are shown by dotted lines.

Potentiation of the effects of histamine on other test objects

The effect of histaminase inhibitors was tested on three other test objects known to give a reproducible and graded response to histamine. The histamine contractions of the guinea-pig uterus and trachea were markedly potentiated by histaminase inhibitors. On the other hand, potentiation of the depressor effect of histamine on the cat's blood pressure was seen only in a minority of experiments.

Guinea-pig tracheal chain. Amongst all preparations investigated the tracheal chain of the guinea-pig gave the strongest and most consistent potentiations with histaminase inhibitors. This is probably due partly to a high histaminase content (p. 44) and partly to the slow rate of contraction of this preparation allowing the enzyme more time to act. A contraction in this preparation takes 10–15 min as compared with 10–15 sec for the ileum. Histaminase inhibitors B_1 pyrimidine or semicarbazide, were added to the bath 4 min before adding

histamine; they produced no effect of their own but caused a marked potentiation of histamine which in some cases amounted to the equivalent of an eightfold increase in dose. The potentiating effect of 10^{-7} and 10^{-6} B_1 pyrimidine is

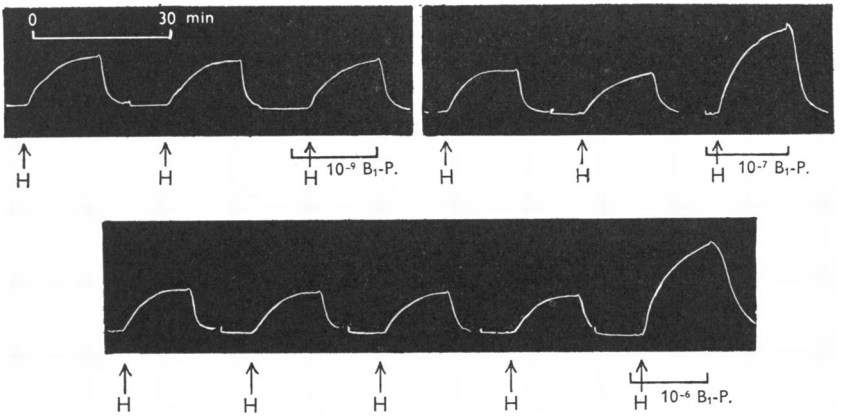


Fig. 6. Potentiation of histamine contractions of guinea-pig tracheal chain in Krebs's solution. Contractions produced by 2×10^{-6} histamine (H). B_1 pyrimidine was added to the bath 4 min before the histamine dose and left in for the period indicated. A concentration of 10^{-9} is sub-threshold; 10^{-7} shows marked potentiation, and 10^{-6} maximum potentiation.

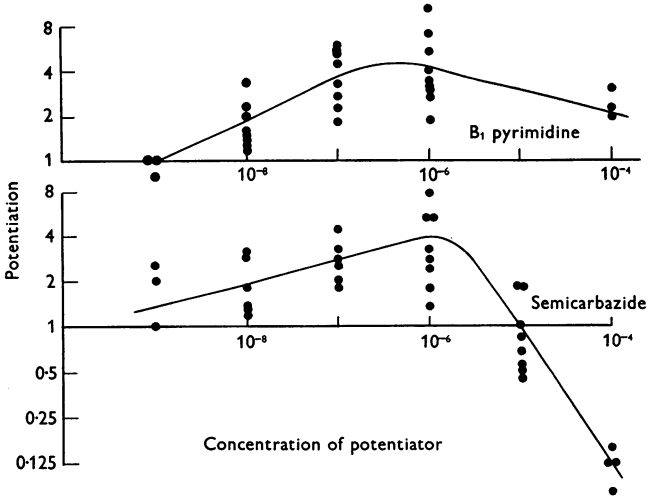


Fig. 7. Effect of concentration of potentiator on histamine contractions of guinea-pig tracheal chain using B_1 pyrimidine and semicarbazide.

illustrated in Fig. 6; it can be reversed by removing the potentiator and can be elicited repeatedly in the same preparation. Fig. 7 illustrates the relation between concentration of B_1 pyrimidine and degree of potentiation. A concentration of 10^{-8} B_1 pyrimidine is already markedly effective; 10^{-6} produces

maximal potentiation; there is little evidence of depression even at 10^{-4}. Semicarbazide, on the other hand, is depressant when used in high concentrations; it potentiates at a concentration as low as 10^{-9} and antagonizes histamine in concentrations above 10^{-5}.

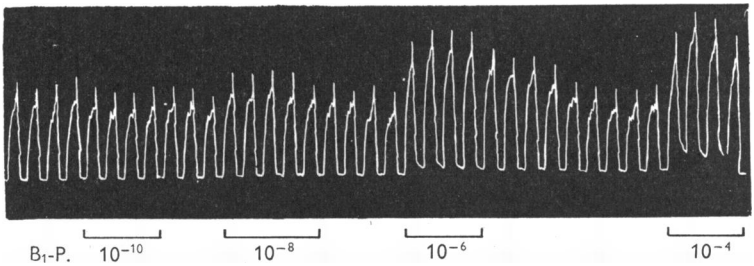


Fig. 8. Potentiation of histamine contractions of guinea-pig uterus in Tyrode solution by B_1 pyrimidine. Contractions were produced by 0·2 μg histamine in a 5 ml. bath. B_1 pyrimidine was added for the periods indicated. A concentration of 10^{-10} has no effect; 10^{-8} is threshold, and 10^{-6} optimum; 10^{-4} causes a rise of base-line.

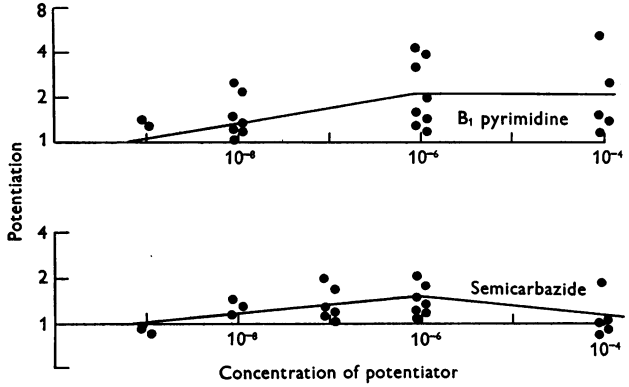


Fig. 9. Effect of concentration of potentiator on histamine contractions of guinea-pig uterus using B_1 pyrimidine and semicarbazide.

Isolated guinea-pig uterus. This preparation is difficult to stabilize, but once stabilized a potentiation of histamine effects by histaminase inhibitors can usually be demonstrated. This is illustrated for four concentrations of B_1 pyrimidine in Fig. 8. Although low concentrations of this compound have no stimulant action of their own, concentrations above 10^{-6} tend to produce a rise of base-line in this preparation. Fig. 9 shows that B_1 pyrimidine produces threshold effects at 10^{-9} and maximal potentiation at 10^{-6} to 10^{-4}. Semicarbazide is less active in this preparation than B_1 pyrimidine.

Cat's blood pressure. Using eviscerated cats under pentobarbitone a distinct potentiation of the depressor effects of histamine by B_1 pyrimidine was recorded

on ten different occasions in four cats; in the remaining seven cats, no potentiation was observed. Instances of potentiation are shown in Fig. 10; in each case B_1 pyrimidine itself caused a fall of blood pressure followed by potentiation of the effects of one or two subsequent doses of histamine. It is interesting that depression of blood pressure by the histaminase inhibitor itself and subsequent potentiation of histamine effect generally occurred together; there was a posi-

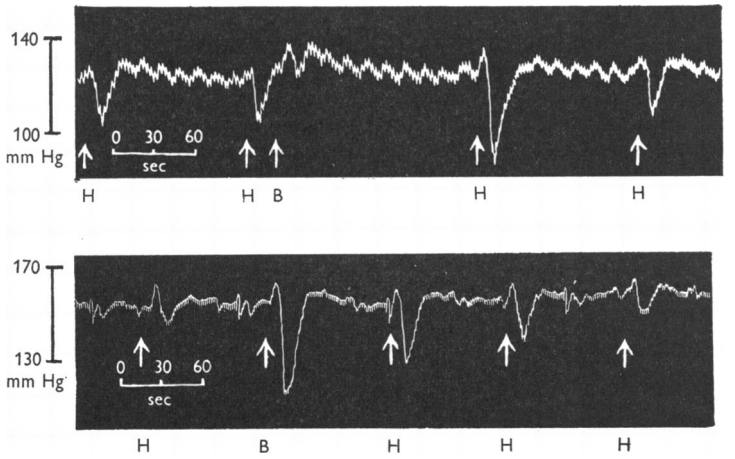


Fig. 10. Potentiation of histamine effects on cat's blood pressure (pentobarbitone, eviscerated) by B_1 pyrimidine. Upper tracing: H, successive injections of 0·13 μg histamine; B, injection of 0·13 mg B_1 pyrimidine has a small depressing action of its own and the effect of the subsequent dose of histamine is potentiated. Lower tracing: H, successive injections of 0·05 μg histamine. B, injection of 0·13 mg B_1 pyrimidine has a large depressing action and the effects of the two following doses of histamine are potentiated.

tive correlation between the magnitude of the two effects ($r = 0{\cdot}74$, $P = < 0{\cdot}01$). In several instances B_1 pyrimidine caused neither depression of blood pressure nor potentiation. Possibly both effects are due to inhibition of histaminase causing in the one case activation of endogenous histamine, and in the other potentiation of injected histamine.

Part II. Potentiation and histaminase inhibition

The most obvious explanation of the potentiating effects of antihistaminases is that they are due to inhibition of the enzyme. But we have not yet excluded the possibility that the potentiation is due to a direct sensitizing effect resulting in a general increase in response to drugs. Three types of experiments were carried out to show that potentiators act by inhibiting histaminase: (1) demonstration that tissues which gave potentiation effects contain histaminase; (2) quantitative comparison of potentiating and antihistaminase activities of different compounds; (3) comparison of the effect of antihistaminases on histamine and on drugs which are resistant to histaminase.

Histaminase content of isolated guinea-pig tissues

The histaminase content of the ileum, trachea and uterus of the guinea-pig was determined. The mucosa of the ileum was scraped off and discarded since preliminary experiments showed that it had a high histaminase content. The trachea was used with cartilage but the fraction of cartilage (about 65 %) was allowed for in the calculation of the enzyme content. Small non-pregnant uteri were used, similar to those used in the pharmacological experiments.

TABLE 2. Percentage destruction of histamine (10^{-7}) by guinea-pig tissues incubated for varying times. Each result is the mean of two or three experiments. The tissue fragments of ileum and uterus occupy 1/100th, and those of trachea 1/300th of the volume of the solution (see text).

	Incubation time (min)					(*a*) Half-time (min) for solution	(*b*) Calculated half-time (sec) for tissue
	30	60	90	120	180		
Trachea	13	19	34	38	48	195	39
Ileum	27	38	57	65	—	78	47
Uterus	7	23	34	45	72	128	77

(It was found that pregnant uteri contained increased amounts of histaminase.) Table 2 shows the rate at which histamine was destroyed in 10 ml. of solution when incubated with about 100 mg of tissue fragments. All three tissues contained appreciable amounts of histaminase, the average half-times found by interpolation were 78, 128 and 195 min for ileum, uterus and trachea, respectively. Under the conditions of these experiments most of the cells are undamaged and it can be assumed that the destruction of histamine takes place mainly in the tissue fragments. The times in Table 2, column '*a*', relate to the histamine depletion in the solution, but for correlating potentiation with destruction of histamine we are interested in the rate of depletion in the tissue fragments which occupy only a small fraction of the solution ($\frac{1}{100}$th for ileum and uterus and $\frac{1}{300}$th for trachea), and in which the rate of destruction must be correspondingly faster. The calculated half-times within the tissue fragments are thus $\frac{195 \times 60}{300} = 39$ sec for trachea, 47 sec for ileum and 77 sec for uterus. Even these times are probably overestimations since the rate of destruction is limited by the rate of diffusion into the fragments.

Correlation between histaminase-inhibiting activity and potentiation

Most of the potentiators we used have previously been described as inhibitors of diamine oxidase or histaminase, but no comparative data were available. It was accordingly decided to determine by a common method the inhibiting activity of a number of potentiators with a view to correlating this with their

potentiating activity. Eight compounds selected from different chemical groups were used in this series. The amount of inhibition of destruction when a histamine solution was incubated with a crude histaminase preparation was determined for a range of concentrations of the inhibitors. By plotting the percentage inhibition of histamine destruction against log. concentration of inhibitor, S-shaped curves were obtained from which the concentration producing a given percentage inhibition may be read off. The inhibition obtained with various concentrations of inhibitors is shown in Table 3.

TABLE 3. Details of inhibition of histaminase activity by eight of the potentiators of Table 1. Substrate: 10^{-6} histamine, incubated at 37° C for 30 min with 10 mg/ml. pig kidney cortex acetone-dried powder.

	% inhibition at various concentrations (M)					
Inhibitor	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
Semicarbazide	10	20	70	81	76	—
Hydroxylamine	—	7	19	68	100	98
Thiosemicarbazide	—	0	2	−5	65	65
Cadaverine	2	−1	16	13	35	56
B_1 pyrimidine	0	22	75	89	86	—
Guanidine	—	1	1	16	53	75
Dimethylguanidine	—	5	4	31	66	93
Iminazole	—	−1	9	29	73	87

The results are summarized graphically in Fig. 11, which illustrates both the potentiating activities and the antihistaminase activities of the eight compounds tested. There is close parallelism between the two types of activity. The two most active potentiators, semicarbazide and B_1 pyrimidine, are the most active inhibitors of histaminase; the guanidine derivatives which are 100 to 1000 times less active as potentiators are correspondingly less active as inhibitors. The remaining compounds are intermediate in both respects. A diagram illustrating the correlation between potentiation and inhibition is shown in Fig. 12. The correlation has been calculated for threshold effects (5 % potentiation and inhibition) and for large effects (40 % potentiation and 50 % inhibition); both correlations are statistically significant.

Specificity of potentiation by antihistaminases

Two groups of drugs not destroyed by histaminase were investigated: (*a*) acetylcholine and choline, (*b*) pyridylethylamine and pyrazolethylamine, two histamine analogues which proved to be resistant to histaminase.

Acetylcholine and choline

Neither acetylcholine nor choline is potentiated by histaminase inhibitors under conditions in which histamine is markedly potentiated. In the experiment illustrated in Fig. 13*a* contractions of the guinea-pig ileum were induced

alternately by histamine and acetylcholine. B_1 pyrimidine (10^{-6}) added to the Tyrode solution markedly increased histamine contractions without affecting those due to acetylcholine. In other similar experiments previously reported (Mongar & Schild, 1951), semicarbazide (10^{-6}) caused a strong potentiation of histamine without affecting acetylcholine. Thus the histaminase inhibitors

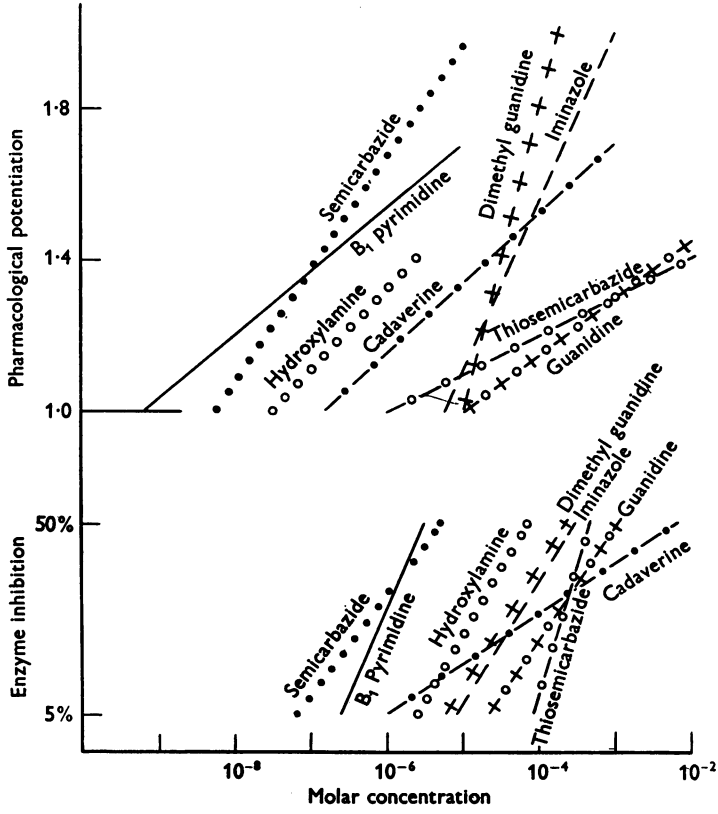


Fig. 11. Comparison of activity of eight compounds as inhibitors of histaminase and potentiators of histamine contractions of the guinea-pig ileum. The slope and position of the lines have been obtained from the data of Table 3 and Figs. 4 and 5. It will be seen that those compounds which are most active as inhibitors of histaminase are also the most active potentiators.

differentiate between histamine and acetylcholine, potentiating only the former. Similar results were obtained using choline instead of acetylcholine. The opposite effect is produced by eserine which preferentially potentiates acetylcholine as shown in Fig. 13*b*. Eserine, however, also causes some potentiation of histamine which may be unspecific and due to the accumulation of endogenous acetylcholine.

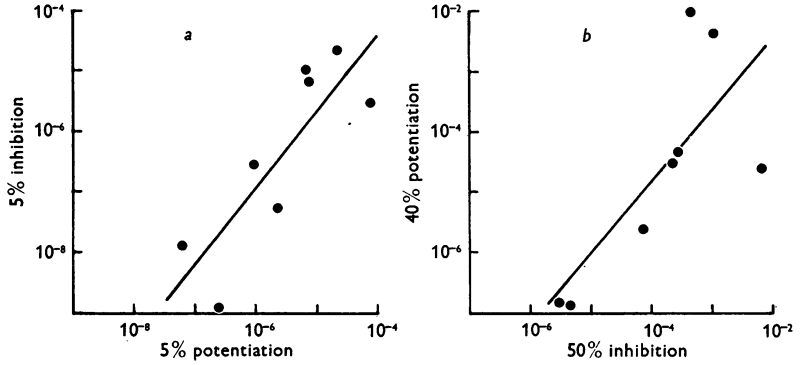


Fig. 12. Correlation between concentrations of eight different compounds required for potentiation of histamine effects on guinea-pig ileum and inhibition of histaminase. (*a*) Concentrations for 5% potentiation and 5% inhibition ($r=0{\cdot}84$, $P=0{\cdot}01$). (*b*) Concentrations for 50% inhibition and 40% potentiation ($r=0{\cdot}89$. $P=0{\cdot}003$). The regression lines have been calculated by least squares.

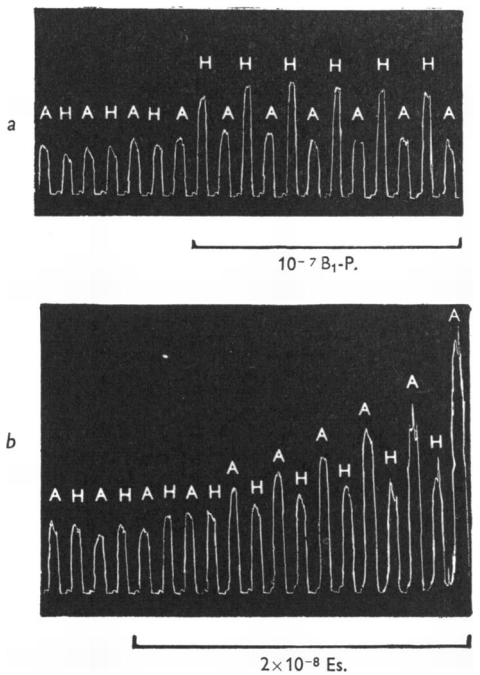


Fig. 13. Specificity of potentiating action. Using alternate doses of acetylcholine 2×10^{-9} (A), and histamine 5×10^{-9} (H), on guinea-pig ileum in Tyrode solution (*a*) 10^{-7} B_1 pyrimidine potentiates only histamine effects (*b*) 2×10^{-8} eserine potentiates mainly acetylcholine effects.

Pyridylethylamine and pyrazolethylamine

β-2-Pyridylethylamine was first investigated by Walter, Hunt & Fosbinder (1941) and β-3-pyrazolethylamine by Lee & Jones (1949). The pyridyl compound had a histamine-like action on smooth muscle and blood pressure, but the pyrazole compound was stated to be devoid of activity on guinea-pig ileum. These compounds have recently been investigated by Arunlakshana (1953) who showed that they both have similar actions to histamine, although higher concentrations are generally required to produce the effects; e.g. they contract the guinea-pig ileum and tracheal chain and lower the cat's blood pressure. Not only have these compounds pharmacological actions which are similar to histamine but they are also antagonized by antihistamines to the same extent as histamine itself. Their pA_2 (Schild, 1947*a*) values against mepyramine and diphenhydramine are the same as those of histamine (Arunlakshana, 1953). These results suggest that these substances act on the same receptors as histamine (Schild, 1947*b*) on smooth muscle. (The word 'receptors' is used here to denote the site of action of the drug and does not imply any theory of its nature; Gaddum, 1943.) There is, however, one distinct difference from histamine: neither compound is destroyed by histaminase as shown in the next section. It was therefore of special interest to find out whether these compounds, which act on the same receptors as histamine but are not destroyed by histaminase, would fail to be potentiated by histaminase inhibitors.

Effect of histaminase. The effect of histaminase on the histamine analogues was tested by two methods. In one series concentrations of the same order of activity of histamine (10^{-7}) pyridylethylamine (10^{-5}) and pyrazolethylamine (5×10^{-5}) were incubated with histaminase, and after a period of 4 hr their activity was assayed biologically on the guinea-pig ileum. The activity of the two analogues was unchanged, whilst most of the histamine had been destroyed.

In another experiment *equal* concentrations of the three compounds were incubated with histaminase, and the ammonia produced was estimated colorimetrically. 12 μg of ammonia was produced by the histamine solution, whilst less than 1 μg was obtained from each analogue. Thus both experiments agree in showing that the two analogues are not destroyed by histaminase.

Effect of histaminase inhibitors. A series of potentiation experiments was carried out with both the pyridyl and pyrazole analogues. The general plan was to administer by means of the automatic apparatus alternate equi-active doses of histamine and one of the analogues, and after a certain time add a histaminase inhibitor to the Tyrode washing solution continuing the administration of alternate doses of the two drugs. This procedure produced a differential potentiation of histamine. Fig. 14 shows absence of potentiation of the pyrazole analogue on the guinea-pig ileum by semicarbazide 10^{-6}, B_1 pyrimidine

10^{-6}, and cadaverine 10^{-5}; in each case the effect of histamine is markedly potentiated. Fig. 15 shows absence of potentiation of the pyridyl analogue on the ileum by semicarbazide 10^{-6} and cadaverine 10^{-5}. Nine experiments of this kind were performed, six with the pyridyl and three with the pyrazole

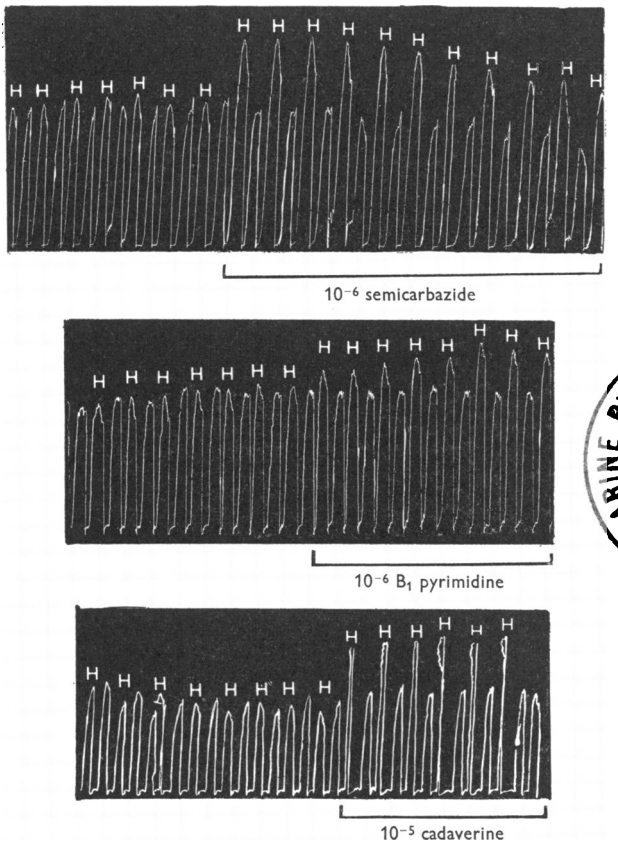


Fig. 14. Specificity of potentiating action using alternate doses of histamine 10^{-8} (H) and pyrazolethylamine $1{\cdot}5 \times 10^{-5}$ (not labelled), on guinea-pig ileum in Tyrode solution. The potentiator was added to the Tyrode solution for the period indicated. In each case only the histamine effects were potentiated.

compound. Differential potentiation could be demonstrated most satisfactorily in preparations newly set up. In these preparations the potentiation was readily reversible. Preparations that had been set up for some time as a rule showed only slight potentiation. When the potentiation experiments were repeated twice or more on the same piece of intestine the effects of the potentiators tended to become irreversible. Under these conditions the effects of histamine and the analogues tended to increase even in the absence of

potentiators and no further potentiation could be achieved by adding the potentiators to the washing solution. In no instance was any potentiation of

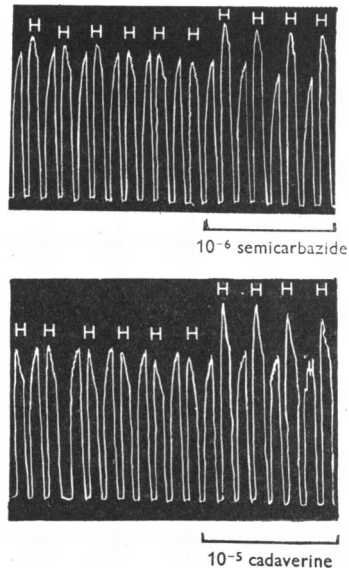


Fig. 15. Specificity of potentiating action using alternate doses of histamine 10^{-8} (H) and pyridylethylamine 5×10^{-7} (not labelled) on guinea-pig ileum in Tyrode solution.

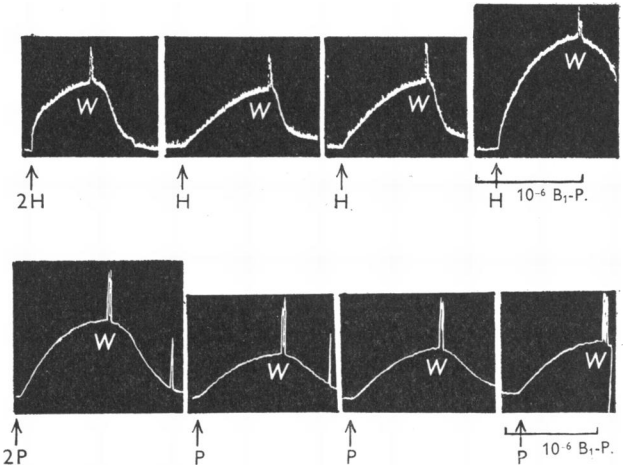


Fig. 16. Specificity of potentiation using guinea-pig tracheal chain in Krebs's solution. H and 2H, contractions produced by histamine concentrations of 4 and 8×10^{-7}; P and 2P, pyridylethylamine concentrations of 2 and 4×10^{-6}. 10^{-6} B_1 pyrimidine was added to the bath for the period indicated. Only the histamine effects were potentiated. Bath washed out at *W*.

the effects of pyridyl- and pyrazolethylamine observed. Corresponding experiments on the tracheal chain are illustrated in Fig. 16. In this preparation the

contrast between the marked potentiation of histamine and the complete absence of potentiation of the stable analogue is particularly clear.

In conclusion, these experiments have shown that compounds which presumably act on the same receptors as histamine, but are resistant to histaminase, are not potentiated by histaminase inhibitors.

DISCUSSION

It has been shown that histaminase inhibitors potentiate histamine effects, and evidence of various kinds has been presented which suggests that the potentiation is due to the inhibition of histaminase. This evidence is as follows:

(1) All histaminase inhibitors so far investigated potentiate histamine effects.

(2) The potentiating activities of different compounds are correlated with their inhibiting activities.

(3) The various tissues in which histamine effects are potentiated contain histaminase; the tissue in which the inhibitors have the greatest effect, the trachea, contains the most histaminase.

(4) Compounds which are not destroyed by histaminase are not potentiated by histamine inhibitors; this also applies to compounds which are closely related to histamine and act in the same way.

The question arises whether the histaminase activity of the tissue is sufficient to account for the observed potentiation. Histaminase activity and potentiation can be correlated as follows. The histaminase activity can be estimated from the experiments in which tissue fragments were incubated with dilute histamine solutions (of the same order of concentration as those used in the pharmacological experiments). The histamine destruction necessary to account for the observed potentiation can be estimated from the degree of potentiation; e.g., since histaminase inhibitors produce a twofold increase of the histamine effect on the ileum, it can be assumed that in the absence of the potentiator only one-half of the histamine on the average reaches the receptors in this preparation, the rest being destroyed by histaminase. The rate of destruction cannot be accurately estimated, but the average time during which it takes place cannot be longer than the contraction time and is presumably less. In ileum this time is 15 sec; within this period one-half of the histamine that would have otherwise reached the receptors has been destroyed. This compares with a half-time for destruction of 50 sec in the incubation experiments.

In the case of the trachea the discrepancy is the other way round. The contraction time is 600 sec for a fourfold potentiation, so that the calculated half-time of destruction in the contracting tissue is 300 sec, whereas the half-time of destruction derived from incubation experiments is only 39 sec. These comparisons are set out in Table 4 for the three tissues concerned; the calculations involved must inevitably be very approximate. For one thing the

histaminase concentration near the histamine receptors may be different from the average activity found for the whole tissue; nothing is known of the localization of histaminase in relation to the receptors. Moreover, as we have seen, both the calculated half-times of destruction have probably been overestimated. But it appears that the enzyme activity is of the right order for the observed potentiations in each case; a better agreement can hardly be expected in view of the assumptions involved, and it is possible that it is only as good as this due to compensation of errors.

TABLE 4. Comparison of calculated half-times for histamine destruction in tissues based on results from potentiation and incubation experiments

Tissue	Maximum potentiation	Contraction time (sec)	Potentiation half-time (sec)	Incubation half-time (sec)
Trachea	Fourfold	600	300	39
Ileum	Twofold	15	15	47
Uterus	Twofold	60	60	77

The reason for the frequent absence of potentiation on the cat's blood pressure is not clear. It may be lack of histaminase but although plasma histaminase in the cat is generally low (Carlsten, 1950; Carlsten & Wood, 1950), there is no information on the histaminase content of the blood vessels themselves.

These compounds may throw some light on possible physiological functions of histamine in the same way as anticholinesterases have helped to elucidate the physiological role of acetylcholine. The evidence for the existence of histaminergic nerves is much more insecure than that for cholinergic nerves, but various scattered observations in the literature suggest that histamine may be the chemical transmitter at some nerve endings. A number of nerves have been found to contain appreciable amounts of histamine (Kwiatkowski, 1943; Euler, 1948; Werle & Weicken, 1949); the histamine content of postganglionic sympathetic fibres has been shown to be particularly high (Rexed & Euler, 1951). Several authors have studied histamine release from stimulated nerves. Positive results have been reported for sympathetic nerves by Lambert & Rosenthal (1940) and Euler & Aström (1948), and for sensory nerves by Ungar (1937) and Kwiatkowski (1943). However, the histamine content of nerves does not diminish after degeneration (Kwiatkowski, 1943; Werle & Weicken, 1949), which suggests that histamine in nerves may have no functional significance. In the present state of uncertainty it would be of interest to find out whether histaminase inhibitors potentiate the effect of nerves believed to be histaminergic and whether they cause an increase in the amount of histamine released.

SUMMARY

1. Histamine contractions of the guinea-pig ileum were potentiated by semicarbazide. Using automatic assay apparatus potentiation has been quantitatively determined at various concentrations of semicarbazide; 10^{-9} produced threshold potentiation, 10^{-6} maximum potentiation (about two-fold), and 10^{-4} depression.

2. Ten other histaminase inhibitors, including carbonyl reagents, diamines, guanidine and iminazole derivatives, also potentiated histamine effects on the guinea-pig ileum.

3. Histaminase inhibitors potentiated the effects of histamine on the isolated guinea-pig tracheal chain and uterus. The tracheal chain gave the greatest potentiation—up to eightfold. The effects of histamine on the cat's blood pressure were not consistently potentiated.

4. The trachea, ileum, and uterus of the guinea-pig contain histaminase.

5. The histaminase-inhibiting activity of eight compounds was quantitatively studied. There was a significant correlation between the concentrations required to produce enzyme inhibition and pharmacological potentiation.

6. Potentiation by histaminase inhibitors was confined to histamine. No potentiation occurred with choline, acetylcholine, or histamine analogues resistant to histaminase.

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