Brit. J. Pharmacol. (1960), **15**, 561.

EVALUATION OF ANTAGONISTS OF HISTAMINE, 5-HYDROXYTRYPTAMINE AND ACETYLCHOLINE IN THE GUINEA-PIG

BY

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(RECEIVED AUGUST 5, 1960)

Changes in the resistance to entry of air into the lungs of anaesthetized guinea-pigs have been used to study the effects of histamine, 5-hydroxytryptamine, and acetylcholine. Some known antagonists of these bronchoconstrictor agents have been investigated. The degree of antagonism has been expressed in terms of the dose ratio, previously used only with *in vitro* preparations.

In 1940 Konzett and Rössler described a simple method of recording the degree to which the lungs of anaesthetized animals resist inflation. Since then the method has been used on many occasions to study physiological changes in resistance, the effects of drugs upon resistance and the action of drugs upon responses produced by agents known to change resistance.

Many workers have assumed that any change in resistance was produced by changes in bronchiolar tone, and this point has been discussed by Konzett (1956). In the present paper a more detailed study is made of the increased resistance to inflation produced in anaesthetized guinea-pigs by histamine, 5-hydroxytryptamine and acetylcholine and of the effects upon these responses of some of the antagonists of these drugs. A method for estimating the potency of these antagonists using the dose ratio (Gaddum, Hameed, Hathway and Stephens, 1955) has been employed.

METHODS

Although the method used was originally evolved for this study it has since been described in detail by Collier, Holgate, Schachter, and Shorley (1960). Resistance to the entry of air into the lungs of guinea-pigs anaesthetized with urethane (1.25 g./kg.) injected intraperitoneally was recorded by the Konzett and Rössler (1940) apparatus connected to the tracheal cannula, the air being supplied by a miniature Starling pump. After the resistance to inflation had been increased by a drug, it was returned to the original level by clipping for 10 sec. the tube leading to the recording apparatus, thus forcing the full stroke volume of the pump into the lungs. This reopened collapsed lung segments. Constancy of the base line resistance was obtained by small alterations in pump stroke volume. Anaesthesia was maintained by further intraperitoneal doses of 0.1 g. urethane. Drugs dissolved in normal saline were given either into the jugular vein in volumes of 0.05 to 0.4 ml. washed in with 0.5 ml. saline containing 10 i.u. heparin/ml. or by stomach tube in volumes up to 10 ml. washed in with 2 ml. saline.

Histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate and acetylcholine chloride were used as agonists. As antagonists, chlorpheniramine maleate, diphenhydramine hydrochloride, mepyramine maleate, 2-bromolysergic acid diethylamide bitartrate, lysergic acid diethylamide tartrate and atropine sulphate were employed. All doses are given in terms of the active acid or base.

RESULTS

Agonists

As a preliminary to experiments using antagonists, control experiments were carried out in which only the agonists were given. With all 3 agonists the response increased over the first 3 to 6 doses, and these were excluded from subsequent analyses and from times given for the duration of experiments. In these control experiments 4 doses in geometric progression were chosen to cover the range of recordable response. These were given at intervals of 5 to 10 (usually 8) min. in random order over a period of $5\frac{1}{2}$ to $6\frac{1}{2}$ hr. with intervals of 30 to 60 min. interpolated between some of the sets of 4 doses.

The response measured in all experiments was the maximum excursion of the piston recorder of the Konzett and Rössler apparatus. This was taken to indicate the maximum resistance to air entry. Preliminary investigations had shown this

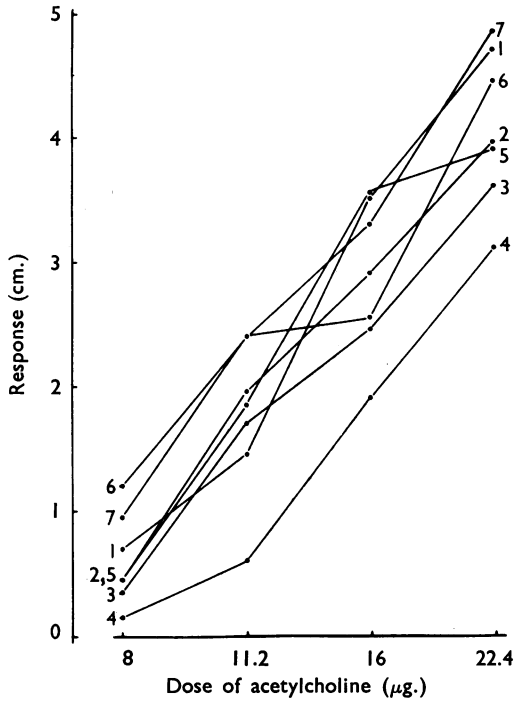

FIG. 1.—Log. dose-response curves for a control experiment with acetylcholine. Each line is drawn through points corresponding to 4 consecutive doses, and numbers indicate order of administration of each set of doses. Intervals of 30–60 min. were interpolated between sets 4, 5, 6, and 7. Responses were measured as the maximum excursion of the piston recorder in cm.

measure to be as informative as the area under the curve relating increase in excursion and time. The response was plotted against log dose of agonist as shown for an experiment on acetylcholine in Fig. 1.

Five such control experiments were carried out, 2 using histamine, 1 using 5-hydroxytryptamine and 2 using acetylcholine. From analysis of the results of these experiments it was found that, with 5-hydroxytryptamine and acetylcholine, the average dose-response curve was linear within the limits of experimental error. The deviations from linearity were significant in the experiments with histamine, but the biasing effect of this on the dose ratio (*vide infra*) was estimated as less than 4%.

In one experiment with histamine the slopes of the dose-response curves varied significantly as the experiment progressed, but in the remaining experiments with histamine and other agonists, differences in slope from one set of 4 responses to another were not significant.

In all control experiments the average level of response varied significantly between sets of responses. The degree of variation was expressed by determining the dose ratio for each set of 4 responses relative to the first set. Ideally this value should be unity since no antagonist had been given. The values obtained for the dose ratio are plotted against time in Fig. 2. In experiments involving acetylcholine the dose ratio showed progressive increase over a series of 4 sets of 4 doses with 8 min. intervals between doses and sets. In some preparations interpolation of

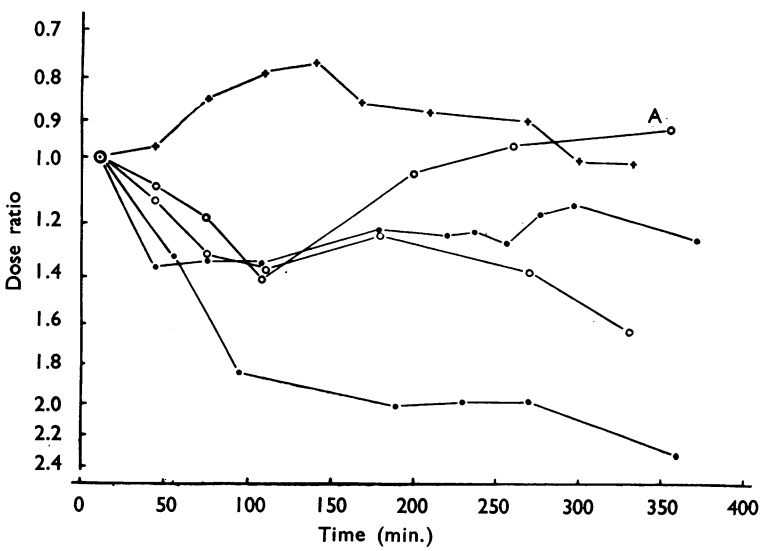

FIG. 2.—Control experiments. Dose ratios of sets of 4 responses relative to first set of 4 responses, plotted against time, taken as the mean for the period covered by a set of 4 observations. ●—●=Histamine; +—+=5-hydroxytryptamine; O—O=acetylcholine. Line A represents the results from the experiment illustrated in Fig. 1.

a 30 or 60 min. interval between 2 consecutive sets led to a decrease in dose ratio. Such a rest period did not, however, lead to a decrease in dose ratio in experiments with histamine.

Antagonists

Following Gaddum *et al.* (1955) the potency of an antagonist at a given concentration and a given time after administration is defined as the ratio A/A_0, where A_0 is the dose of the agonist producing a response (y) before the antagonist, and A is the dose of the agonist producing the same response (y) at the given time after the antagonist is administered. The dose ratio can be defined unambiguously only when the log. dose-response curves before and after giving the antagonist are parallel.

To obtain suitable data for the calculation of the dose ratio thus defined the following experimental procedure was adopted. Two doses of agonist were given repeatedly until the responses were stable. The antagonist was then given and the rise and fall of effect was followed by giving increasing and decreasing doses of agonist. Pairs of doses were chosen which, it was hoped, would produce responses comparable in magnitude to those obtained in the absence of antagonist. During the decline in the degree of antagonism intervals of up to 1 hr. were often allowed to elapse without doses of agonist being given. In the case of acetylcholine this was necessary in order to obtain maximal degree of recovery of the preparation (*vide supra*). When the dose ratio had fallen to 2 or less and stable responses were obtained to 2 doses of agonist, a further dose of antagonist was sometimes given.

From the data thus obtained the dose ratio could be determined graphically or by computation. The graphical method, suitable for routine investigation of antagonism, consists of plotting the responses against log. dose, estimating by eye the average slope within pairs of responses and drawing parallel lines through the centroid of each pair. The log. of the dose ratio is given by the horizontal distance between the parallel lines.

The computational approach, which is less subjective, has been used in all experiments reported here. The average slope within sets (usually a pair) over the duration of the experiment was calculated by standard regression procedures and the dose ratio determined from parallel lines with this slope fitted to sets of comparable responses.

In both methods where the degree of antagonism changes rapidly with time, individual responses may have to be used.

TABLE I

ANTAGONISM OF 5-HYDROXYTRYPTAMINE BY LYSERGIC ACID DIETHYLAMIDE

At 28 min. 50 μg./kg. lysergic acid diethylamide was given intravenously.

Dose No.	Time (min.)	Dose of 5-Hydroxy-tryptamine (μg.)	Response (cm.)
1	0	1	2·1
2	8	2	3·9
3	16	2	3·7
4	24	1	1·25
5	32	10	1·4
6	40	20	1·6
7	48	40	3·1
8	56	40	2·0
9	64	20	1·0
10	124	20	4·4
11	132	10	2·35
12	192	10	4·05
13	200	5	2·65
14	290	5	4·6
15	298	2·5	3·7
16	306	4	4·3
17	314	1	1·05
18	322	2	2·1

Table I gives the results of an experiment with 5-hydroxytryptamine and lysergic acid diethylamide; and Fig. 3 illustrates the method of determining from these data the dose ratio at different times.

Table II shows for 6 chosen antagonists dose ratios which are the maximal calculated during each experiment. When the antagonist was given intravenously the degree of antagonism often changed rapidly in comparison with the interval of 8 min. between doses of agonist. This may have resulted in underestimation of the maximal dose ratio.

Specificity of Antagonism

Specificity of antagonism was examined using diphenhydramine, mepyramine, lysergic acid diethylamide and atropine. Pairs of doses of each of the 3 agonists—histamine, 5-hydroxytryptamine and acetylcholine—were given at 5 min. intervals, usually in serial order with high and low doses alternating. When stable responses to each of these doses were obtained the antagonist was given intravenously. The sequence of doses of agonist was continued, increasing them where necessary to give responses

TABLE II

DOSE RATIOS CALCULATED FOR SIX ANTAGONISTS AT VARYING DOSES

Agonist	Antagonist	Dose (μg./kg.)	Route	Dose Ratio	No. of Experiments	Time at which Dose Ratio Measured (min.)
Histamine	Chlorpheniramine	1·6	Intravenous	1·5	1	4
		4		1·6	1	16
		16		3·1	1	4
		32		2·8	1	8
		40		5·4	1	12
		77		16	1	32
		400		27	1	32
		400	Oral	3·2	1	24
		1,700		52–330	3	44–80
	Diphenhydramine	3–6	Intravenous	0·8–1·4	4	8–32
		33–40		1·3–1·6	3	8–16
		80		1·8	1	4
		165		3·7	1	16
		320–400		3·0–6·8	4	8–28
		800		6·6	2	4–12
		2,000		32	1	24
		1,700–1,800	Oral	1·3–2·7	2	32–48
		17,000–18,000		40–62	3	64–84
	Mepyramine	4	Intravenous	2·4–4·2	2	12–24
		40		12–28	3	24–40
		400		3,500	1	32
		1,750–1,800	Oral	2.0–9·0	4	56–80
		8,750–9,000		12–110	3	40–104
		17,500		730	1	88
5-Hydroxytryptamine	Bromolysergic acid diethylamide	1	Intravenous	0·9–1·0	3	8–16
		10		1·9–6·2	3	16–24
		100		10–23	3	24–40
		100	Oral	1·0	1	16
		400–500		1·9–2·8	2	56–102
		2,000		3·9–4·9	2	72–160
	Lysergic acid diethylamide	1	Intravenous	1·0–2·5	3	16–32
		10		4·8–7·9	3	24–32
		50		32–34	2	32
		100		25–55	3	32–40
		50	Oral	6·1–14	2	32–88
		100		15	1	100
Acetylcholine	Atropine	1	Intravenous	1·1–1·3	2	8
		10		3·7-4·2	2	8–12
		100		10–20	2	4–8
		1,000	Oral	3·0–6·3	2	20–50
		5,000		22	1	188
		10,000		49	1	186

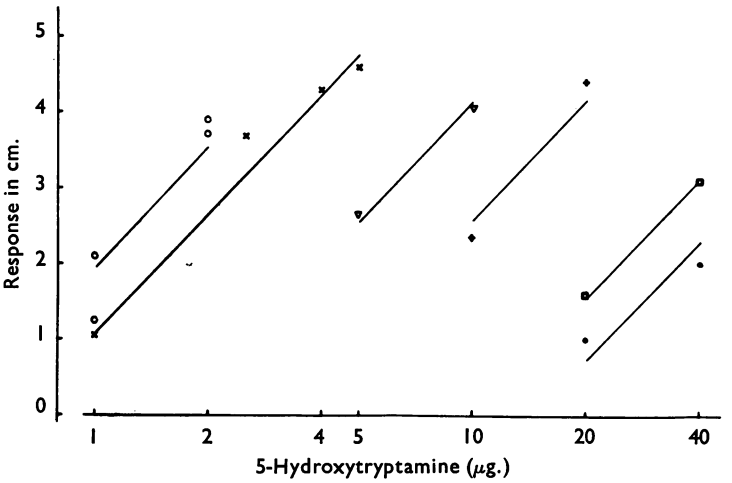

FIG. 3.—Log. dose-response curves illustrating data of Table I. O—O=Responses 1 to 4; □—□=responses 6 and 7; ●—●=responses 8 and 9; +—+=responses 10 and 11; ▽—▽=responses 12 and 13; and X—X=responses 14 to 18. For the 5 sets of responses after lysergic acid diethylamide the dose ratios were 24, 34, 7.6, 3.8, and 1.5 at 16, 32, 100, 168, and 278 min. respectively after giving the antagonist.

TABLE III

DOSE RATIOS OBTAINED IN SPECIFICITY EXPERIMENTS

Dose ratios given are the maxima or minima observed.

Antagonist	Intravenous Dose (μg./kg.)	Histamine		5-Hydroxytryptamine		Acetylcholine	
		Dose Ratio	Time (min.)	Dose Ratio	Time (min.)	Dose Ratio	Time (min.)
Diphenhydramine	400	7·8	37·5	1·9	12·5	2·0	102·5
Mepyramine ..	40	15	12·5	0·98	25	1·6	37·5
Lysergic acid diethylamide ..	50	0·83	15	53	32·5	0·76	10
Atropine ..	100	2·8	22·5	1·4	12·5	18	27·5
,, ..	100	2·4	10	1·7	15	27	20

similar to those obtained in the absence of the antagonist. The dose ratios could then be calculated for each agonist independently. The results are shown in Table III, in which dose ratios of less than 1 indicate potentiation. The 2 experiments with atropine showed good agreement.

DISCUSSION

This work shows that methods of evaluating the potency of an antagonist previously used *in vitro* can be applied to an *in vivo* preparation provided that the response to the agonist remains sufficiently stable over an adequate period of time. We believe that this has been achieved by certain refinements of the technique first reported by Konzett and Rössler (1940). These include keeping the preparation at a fairly constant degree of anaesthesia and maintaining aeration near the original level by reinflation of collapsed lung segments and slight alterations in pump stroke volume.

Sufficient stability of response was indicated in the control experiments previously described and illustrated in Fig. 1. The greatest loss of response resulted in a dose ratio of 2.3 over a period of 6 hr. Over shorter periods of time corresponding to times of maximal antagonism in Table II the change of response was always considerably less. The evidence of these control experiments is supported by many experiments in which antagonists have been given and full recovery of response observed after 5 to 6 hr. when the effects of the antagonists have disappeared.

When an antagonist has been given, the slopes of the log. dose-response curves before and after its administration have usually been comparable, the exception being when high doses of lysergic acid diethylamide were used. In this case the slope was decreased and it was impossible to calculate dose ratios.

The results given in Table II provide a basis for studying the variation in dose ratio between

animals given the same dose of antagonist. This variation is best measured by the coefficient of variation which is estimated from these results as about 35% for drugs given intravenously and about 70% for those given by mouth. Presumably this larger figure reflects variation in absorption of the antagonist.

From these results in an *in vivo* preparation, we can study the relative potency of a drug given intravenously and by mouth. In this short series of drugs remarkable differences can be seen as shown in Table IV. Even among the 3 antihistamines and the 2 antagonists of 5-hydroxytryptamine great differences exist.

TABLE IV

RATIOS OF ORAL TO INTRAVENOUS DOSES GIVING EQUAL DEGREES OF MAXIMAL ANTAGONISM

Antagonist	Approximate Ratio
Chlorpheniramine	4
Diphenhydramine	6
Mepyramine	200
Bromolysergic acid diethylamide..	100
Lysergic acid diethylamide ..	4
Atropine	60

In the specificity experiments given in Table III, dose ratios obtained against the primary agonist are reasonably comparable with those given in Table II, indicating that the use of other agonists in these experiments does not modify the primary antagonism. Both antihistamines showed specificity although this was greater for mepyramine than for diphenhydramine. Both antagonized acetylcholine to some extent, but the maximum dose ratio was obtained at a later time than that for histamine. The results for lysergic acid diethylamide were interesting in that the compound appeared to potentiate histamine and acetylcholine. This finding confirmed a previous experiment in which the responses before and after lysergic acid diethylamide were not sufficiently comparable to enable the dose ratio to be calculated.

In the 2 experiments using atropine, both histamine and 5-hydroxytryptamine were antagonized to some extent though the dose ratio is rather greater for histamine. This contrasts with the finding of Cambridge and Holgate (1955), using guinea-pig ileum, and suggests that 5-hydroxytryptamine is probably not acting by the liberation of acetylcholine. One would speculate that the 2 types of receptor suggested by Gaddum and Picarelli (1957) are not present in the bronchioles.

We thank Dr. H. O. J. Collier for his helpful suggestions and acknowledge the assistance of Misses L. S. Deeming and C. H. Franco and Messrs P. A. Berry, L. C. Dinneen and M. Summersfield.

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