Quantitative studies in humans on the antagonism of lysergic acid diethylamide by chlorpromazine and phenoxybenzamine

In human volunteers, 198 trials were made to determine the threshold dose for recognition of lysergic acid diethylamide (LSD-25) and the effect upon this of chlorpromazine and phenoxybenzamine. The threshold dose of LSD was determined to be 20 μg or 0.260 to 0.295 μg per kilogram. Chlorpromazine, in a dose of 25 mg., given 30 minutes beforehand or phenoxybenzamine, in a 10 mg. dose, given at the same time blocked recognition of this dose of LSD, i.e., raised the threshold. The same dose of chlorpromazine when given at the same time as or 30 minutes after LSD did not block this recognition. Neither chlorpromazine in this dosage nor phenoxybenzamine in doses up to 30 mg. had any significant physiologic or subjective effect. The results suggest that the mechanisms through which these compounds block the recognition of LSD are different from those producing their peripheral autonomic effects.

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Critical evaluation of antagonism in humans of one drug by another is frequently quite difficult. In dealing with drugs which affect behavior, the problems of subjectivism and suggestibility strongly enter the picture. Furthermore, since the methods used in various studies are usually quite different, it is often impossible to compare one set of investigations with another; thus, seemingly conflicting reports often appear. A uniform method for gauging antagonism would be valuable not only because this might provide a means of searching for clues to new therapeutic compounds but, more importantly, because it would provide a means of gaining insight into basic mechanisms of action.

Lysergic acid diethylamide (LSD-25) is of interest because it is highly potent and specific in the sense that distinct subjective effects follow doses which do not produce marked physiologic effects. Most reports on the actions of LSD in humans have been in terms of some variety of subjective scaling on the part of subjects or observers or both. Even in carefully controlled double blind settings, the influence of bias may be substantial. Adequate statistical analysis of results of subjective scaling is complex.

Many agents have been reported to an-

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tagonize LSD in humans. Among these, chlorpromazine has been investigated most. It has been reported to act against LSD when given before,[11] after,[6, 10, 12, 13] or both before and after the latter.[4, 8, 15] It also has been reported to be ineffective against LSD[3] and even to potentiate the effects of LSD.[1] Isbell[7] reports that it is effective against LSD when administered either before or after, but much more effective before than after; according to his findings, if chlorpromazine is given after LSD, the parenteral route must be used.

Any route of administration will itself be a source of unreliability, to some degree. The parenteral route usually, though not always, is more reliable than the oral as far as absorption is concerned. Unfortunately, it also is much more likely to elicit responses determined by emotion rather than by drug. The oral route can be reduced to a standardized routine with a minimal emotional component which presumably cancels out when the Latin square experimental design is followed.

In the studies reported here, threshold doses of LSD were administered orally to trained subjects. The subjects could then respond with a simple statement as to whether they were given an active compound, thus obviating the special problems with subjectivism and complex statistics associated with the use of scales. The use of these small doses minimizes derangement of physiologic condition and also imposes minimal emotional stress upon the subjects, and it may thus serve to reduce artifactual results. Antagonism or synergism can be gauged by whether a second compound raises or lowers the threshold for the compound under study or leaves this threshold unchanged. This simplified, uniform procedure can easily be reproduced in other settings, so that results from one study can be closely compared with those of other studies.

Procedure

The following work was undertaken to determine threshold doses for recognition of LSD by normal humans and to investigate the effect of some other compounds on these thresholds.

Subjects. Eighteen voluntary subjects, inmates of the U. S. Penitentiary, Atlanta, were used. Physiologically, they were within normal limits in medical history and physical examinations. Their intellectual and emotional status was assessed by review of their institution record, U. S. Public Health Service and other intelligence scores, personal interviews, and Rorschach test and Minnesota Multiphasic Personality Inventory results. They all had at least average intelligence; the range of IQs was 96 to 146 on the Wechsler Adult Intelligence Scale. None had gross psychiatric illness, although most suffered from character disorders of appreciable degree.

After this screening, they were given a period of training in the effects of LSD. They received increasing doses at weekly intervals: 25 μg the first week, 50 μg the second, 75 μg the third, and 100 μg the final week; doses larger than 100 μg were not given. For their participation, the subjects received $3 per week plus 3 days of credit toward earlier release for each month they were a part of the experimental program. They were carefully educated to the viewpoint that the results they gave did not in any way affect their status and that the only thing wanted was their frank report of the effect of the compounds. They were free to withdraw any time, although none did so.

Method. The first step in the investigations was to determine what threshold dose of LSD these trained subjects could recognize with statistically significant accuracy. The subjects were given 0, 5, 10, 15, or 20 μg. When the threshold was established, doses of the average number of micrograms per kilogram were administered as a check.

To determine the antagonistic or synergistic effect of chlorpromazine, a dose of 25 mg. was given with blank or 15 or 20 μg of LSD. This dose of chlorpromazine was selected as small enough not to pro-

duce frank sedation, so that any antagonism found need not be attributed to this factor.

Phenoxybenzamine was selected as an adrenergic blocking agent with peripheral effects similar to those of chlorpromazine. Others have found incomplete block of LSD by this compound.[2, 9] It was given in doses of 10, 20, and 30 mg. alone and in doses of 10 and 20 mg. with blank or 15 or 20 μg of LSD.

Finally, 25 mg. of chlorpromazine was given 30 minutes before or 30 minutes after test doses of blank or 15 or 20 μg of LSD. The subjects in this phase of the experiment might receive any of seven pairs of doses:

First dose	*Second dose*
25 mg. chlorpromazine	20 μg LSD
20 μg LSD	25 mg. chlorpromazine
25 mg. chlorpromazine	15 μg LSD
15 μg LSD	25 mg. chlorpromazine
25 mg. chlorpromazine	Blank
Blank	25 mg. chlorpromazine
Blank	Blank

Phenoxybenzamine has not yet been tested in this way. Although these dosages are described in consecutive order for clarity, they were not given in systematic sequence. Rather, a randomized Latin square design was followed, with the limitation that the one dose schedules and the two dose schedules did not overlap. All subjects during a given phase received the same number of doses.

The following measurements were made by one of two physicians before each administration of drug and at hourly intervals afterward: blood pressure, pulse rate, pupil diameter, oral temperature, grip strength, and hand steadiness. The same physician made all measurements for each experimental run.

Blood pressures and pulse rates were measured with the subjects supine. The same arm was used with each subject in all instances. The auscultatory method with the sphygomanometer was used for determining blood pressures. Orthostatic blood pressures taken immediately after the supine pressures at 3 hours were obtained for blank, 25 mg. of chlorpromazine, 10 mg. of phenoxybenzamine, and LSD in all doses. Unfortunately, this measurement was not obtained in adequate numbers of subjects with the larger doses of phenoxybenzamine or the various combinations. Pupil diameters were measured to the nearest millimeter; the physicians who did this had had several months of prior practice. This determination was always made with the subject in the same position in the same part of the same room under the same lighting conditions. Since observations were made before dosage on each experimental run, each group of subjects always served as their own controls for every run.

The subjects gave a verbal report of effects which was recorded verbatim without probing 3 hours after dosage. In addition, each kept a running diary for 24 hours which they then turned in before learning what their dose had been. Hemoglobin, white blood count, and differential determinations and urinalyses were obtained at the end of each test period of 24 hours. Each subject was tested no more often than once a week. The drug was given by the physician under double blind procedure. Latin square design was employed throughout. The study extended over a period of 4 months; 198 complete records were obtained.

The results were analyzed as follows: Blood pressures, pulse rates, oral temperatures, and grip strengths were analyzed by means of f ratios. To illustrate, the control pulse rates were entered in a column. In the next column were the rates taken 1 hour after dosage. In the next were the 2 hour readings, and in the final column were the 3 hour readings. The total variance between the columns divided by the total variance within the columns constitutes the f ratio[14] and is an indication in this case of whether significant change occurred during the period of observation. Differences between supine and orthostatic blood pressures were examined by means of the t

test. The distribution of blood pressures in normal humans is probably skewed; the t test would in this case exaggerate differenences; therefore, if t shows no significant difference, one may accept this with confidence. Hand steadiness was analyzed by means of the chi square test for significance of variations in number of counts per 30 seconds as measured at hourly intervals. The average pupil diameter for each control period was calculated. Diameters within ± 0.5 mm. of this value were rated as "average." Diameters more than 0.5 mm. larger were tallied as "greater"; diameters more than 0.5 mm. less were entered as "smaller." Thus, for each set of hourly observations, there were three observed frequencies; since there were four measurements per experimental run, this yielded a 3 by 4 matrix from which χ^2 was calculated. The subjective responses were entered in 2 by 2 tables. On one axis were the alternatives of whether or not the subject received LSD. On the other were the alternatives of whether or not he rated whatever he received as LSD. Thus, for example, from the data in Table I, the responses of the 15 subjects who received 10 μg of LSD were compared with responses of the 14 who received blank; the total number for this 2 by 2 table was then 29. The subject was considered to have rated the compound as LSD only if he specifically mentioned this drug by name in reporting the effects he experienced. Since there were 2 by 2 matrices with f_0 less than 10 in some instances, the Yates correction was applied in all instances.[5] To test reproducibility of the subjective threshold, a randomly chosen group of the subjects was again given doses of 20 μg of LSD versus blank in the last stages of the investigation. This was in the fourth month after the initial determinations of the threshold.

Results

The threshold dose of LSD as initially determined by this procedure was 20 μg, or an average of 0.260 μg per kilogram. This was distinguished from blank with a value $p < 0.05$ (Table I). Doses of 0.295 μg per kilogram were distinguished from blank with a value $p < 0.01$ ($\chi^2 = 8.74$, df = 1). The 20 μg doses given to test reproducibility were also distinguished significantly from blank with $p < 0.05$ ($\chi^2 = 6.33$). If the results of all three phases in which LSD in doses of 20 μg or 0.295 μg per kilogram was compared with blank are pooled, $\chi^2 = 13.15$ and $p < 0.001$. This is a reflection of the increased number of subjects. This range of dosage produced pupillary dilation ($p < 0.01$) in all cases. No other statistically significant physiologic effect appeared.

Table II shows the interaction of 25 mg. of chlorpromazine with 15 μg of LSD. Chlorpromazine alone in this dosage produced no significant physiologic effect. No significant change in pupil diameter occurred, and there was no significant difference between supine and orthostatic blood pressures 3 hours after dosage (systolic t = 1.26, diastolic t = 2). The subjects reported subjective effects of chlorpromazine as follows:

Like LSD	1	Relaxed	1
Sedative	4	Nervous	1
Stimulant	1	No effect	3

This is a summary of their undirected verbalizations. In no case was the effect very strong. For comparison, here are the subjective effects reported after blank:

Like LSD	4	Blank	8
Sedative	1	Don't know	1
Stimulant	0		

If from these two groups of reports one sets up a 4 by 2 matrix (N = 25) with chlorpromazine versus blank on one axis and on the other axis "like LSD," "sedative," and "blank," versus all other effects, $\chi^2 = 6.44$. With df = 3, this reflects no significant difference ($p < 0.1$) in subjective effect between 25 mg. of chlorpromazine and blank. When 25 mg. of chlorpromazine was given at the same time as or 30 minutes after the 15 μg (0.195 μg per

Table I. *Thresholds for LSD*

Dose			Recognition (χ^2) ($df = 1$)	Pupils (χ^2) ($df = 6$)
μg	μg per Kg.	N		
0 (blank)	0.000	14		6.41
5	0.066	8	1.77	6.35
10	0.136	15	0.91	4.98
15	0.195	11	0.64	7.15
20	0.260	11	3.92*	18.34†

*p < 0.05.
†p < 0.01.

Table II. *Chlorpromazine (25 mg.) versus LSD (15 μg)*

Compounds given	μg per Kg. LSD	N	Recognition (χ^2) ($df = 1$)	Pupils (χ^2) ($df = 6$)
Chlorpromazine (25 mg.) alone	0.000	11		6.13
Chlorpromazine with LSD	0.195	8	5.80*	3.33
Chlorpromazine 30 minutes before LSD	0.198	10	3.40	3.36
Chlorpromazine 30 minutes after LSD	0.204	10	8.10†	1.25

*p < 0.02.
†p < 0.01.

kilogram) dose of LSD, the subjects were able to recognize the latter with $p < 0.02$ and 0.01, respectively. But when it was given 30 minutes before the LSD, the subjects were not able to recognize the same dose of LSD. No pupillary effect or any other physiologic effect appeared in any of these instances.

Table III shows similar results when 25 mg. of chlorpromazine was tried against 20 μg of LSD. Again, only when it was given 30 minutes before LSD did it prevent recognition of the latter. The pupillary dilation found with LSD alone in this dosage was blocked in all instances, however. Once again, no other physiologic effect was obtained.

Phenoxybenzamine alone in doses of 10, 20, or 30 mg. had no statistically significant effect upon any physiologic measurement. No change occurred in pupil diameter. There was no significant difference between supine and orthostatic blood pressures 3 hours after 10 mg. of phenoxybenzamine (systolic $t = 0.58$, diastolic $t = 1.03$). The subjects reported the subjective effects of 30 mg. of phenoxybenzamine as follows:

Like LSD	3	Stimulant	1
Sedative	2	No effect	4

Again, none of these effects were strong. If these reports are compared with those for blank in the same way as those for chlorpromazine versus blank above, $\chi^2 = 1.23$ and $p > 0.7$ ($N = 24$), that is, there is no significant difference in subjective effect between 30 mg. of phenoxybenzamine and blank. However, 10 mg. of phenoxybenzamine given simultaneously with LSD effectively blocked recognition of 20 μg (0.276 μg per kilogram) of the latter (Table IV).

No significant pupillary dilation occurred, nor was any other physiologic effect obtained.

Discussion

The first physiologic effect with very small doses of LSD is dilation of the pupils. Visual changes resulting from this are not the essential cues to the subjects for recognition of the compound, however, since they are still able to recognize it when this effect is blocked by chlorpromazine.

Phenoxybenzamine would seem to be a more effective blocking agent against the recognition of threshold doses of LSD by humans since it is able to block when given simultaneously, even in small doses. This needs further study, however.

Chlorpromazine at this dosage, and by the oral route, must be given before LSD in order to be an effective blocking agent. In larger doses, it might block if given at

Table III. *Chlorpromazine (25 mg.) versus LSD (20 μg)*

Compounds given	*μg per Kg. LSD*	*N*	*Recognition (χ^2) ($df = 1$)*	*Pupils (χ^2) ($df = 6$)*
Chlorpromazine (25 mg.) alone	0.000	11		6.13
Chlorpromazine with LSD	0.259	10	5.82*	2.28
Chlorpromazine 30 minutes before LSD	0.260	10	0.90	2.50
Chlorpromazine 30 minutes after LSD	0.282	10	8.10†	4.41

*$p < 0.02$.
†$p < 0.01$.

Table IV. *Phenoxybenzamine versus LSD*

Doses					
Phenoxybenzamine (mg.)	*LSD*		*N*	*Recognition (χ^2) ($df = 1$)*	*Pupils (χ^2) ($df = 6$)*
	μg	*μg per Kg.*			
10	0	0.000	10		1.66
20	0	0.000	10		5.27
30	0	0.000	10		7.36
10	15	0.189	10	1.88	3.47
10	20	0.276	9	2.53	6.83
20	20	0.280	10	0.20	1.35

All these χ^2 values reflect $p > 0.05$.

the same time or even later than LSD. Each of these drugs has diverse effects which may differ in prominence at varying times after dosage. Possibly herein lies the explanation of the finding that chlorpromazine given at the same time as or after LSD actually lowers the threshold for recognition of the latter.

It may be questioned whether the antagonism of LSD by chlorpromazine and phenoxybenzamine is correlated with their well-known peripheral autonomic effects. In the doses used, neither alone produced any significant autonomic effect, e.g., pulse rate, blood pressure, and pupil diameter.

It should be remembered that these subjects were not representative of the general population sociologically or psychologically. Thus it might eventuate that the findings in a more unselected group would be different.

Conclusion

The threshold dose for recognition of LSD by this group of trained human subjects is about 20 μg or in the range of 0.260 to 0.295 μg per kilogram. This produces significant dilation of the pupils but no other physiologic effect.

A dose of 25 mg. of chlorpromazine does not block this recognition when given at the same time nor when given 30 minutes later; rather, dosage at these times facilitates the recognition of 15 μg of LSD. This dose of chlorpromazine does block if given 30 minutes earlier. The pupillary dilation is blocked in all three instances. This is essentially in agreement with the findings of Isbell.[7]

A dose of 10 mg. of phenoxybenzamine, when given simultaneously, does block the recognition of 20 μg of LSD and also blocks the dilation of the pupils.

Chlorpromazine (25 mg.) and phenoxybenzamine (up to 30 mg.) given alone had no significant effect subjectively or physiologically.

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