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Cross Tolerance Between D-2-Brom-Lysergic Acid Diethylamide (BOL-148) and the D-Diethylamide of Lysergic Acid (LSD-25)

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One of the most striking phenomena observed in clinical studies of the psychotomimetic effects of D-diethylamide of lysergic acid (LSD-25) is the rapid development of nearly complete tolerance (ISBELL *et al.* 1956; ABRAMSON *et al.* 1956) to the "subjective" and "objective" effects when the drug is administered daily. It, therefore, seemed of interest to determine whether pretreatment with D-2-brom-lysergic acid diethylamide (BOL-148), a substance relatively inert as a psychotomimetic agent in man (CERLETTI and ROTHLIN 1955; ROTHLIN 1957; JARVIK *et al.* 1955; ISBELL *et al.* 1959) would attenuate the LSD reaction.

GINZEL and MAYER-GROSS (1956) have already reported some preliminary studies of this sort. They observed no decrease in the subjective effects of LSD when 0.5 to 1.0 mg of BOL-148 was given intravenously at the height of the LSD reaction. When, however, 2 or 3 mg were given during the course of the 24 hours prior to the administration of LSD, partial attenuation of the mental reaction caused by LSD occurred. Following two days of pretreatment with 2 to 3 mg of BOL-148 daily, the reaction induced by 50 to 75 mcg of LSD was almost completely blocked. ABRAMSON *et al.* (1958) noted a partial reduction in the degree of the subjective reaction induced by 50 to 75 mcg of LSD following six to eight days pretreatment with 850 to 1450 mcg of BOL-148 (total dosage) in 4 subjects.

Because of the theoretical importance of possible cross tolerance between BOL-148 and LSD, especially as related to theories ascribing the psychotomimetic effects of LSD to blockade of serotonin, it was felt worthwhile to repeat and extend the experiments of GINZEL and MAYER-GROSS and ABRAMSON *et al.* using methods which would yield information on the "objective" effects of LSD-25 as well as information on the "subjective" effects.

Methods

Subjects. Subjects used for these experiments were all adult negro male former drug addicts who were serving sentences for violation of the U. S. narcotic laws. All subjects volunteered for the experiments,

all were physically healthy, and none presented any evidence of any of the major psychoses. All subjects had received LSD-25 on previous occasions.

General conditions. Experiments were conducted in a special ward devoted to clinical research. Observations were made by especially trained attendants of long experience in observing effects induced by LSD and other types of drugs. In experiments in which BOL-148 was given for two to five days, the patients received the drug three times daily and went about their usual pursuits. On the experimental days, they remained in the wards but were free to mix and mingle with other patients in a common dayroom if they so desired.

Drugs[1] were given orally in solution in 30 cc of cherry syrup. Doses of LSD were calculated on a microgram per kilogram basis. The dose of BOL was the total dose administered irrespective of body weight. Identity of the drugs was unknown to both the patients and observers ("double-blind" procedure).

Observations. The following measurements were made hourly from 6 a.m. to 4 p.m. twice before and eight times after administration of LSD: systolic blood pressure, by the auscultatory method, after the patient had rested quietly in bed for ten minutes; and pupillary size, which was determined under conditions of controlled light and accommodation by comparing the size of the pupils with those of blackened circles of known diameter on a printed card. In addition, the patients completed, with the help of an aide, a modification[2] of the questionnaire of ABRAMSON *et al.* (1955) twice before and $^1/_2$, $1^1/_2$, $2^1/_2$, $3^1/_2$, $4^1/_2$, $5^1/_2$, $6^1/_2$ and $7^1/_2$ hours after administration of LSD. Short mental status examinations were performed at intervals and clinical grades[3] assigned according to the system of ISBELL *et al.* (1956).

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[1] We are indebted to Dr. R. BIRCHER, Sandoz Pharmaceuticals, Hanover, N. J., for supplies of BOL-148.

[2] The questionnaire consists of 57 questions and includes such items as: Are you nervous? Are you confused? Are you having difficulty in thinking? Have you had any peculiar experiences? Do things appear large or small? Is someone controlling your mind, etc? The questionnaire has several disadvantages: it may suggest symptoms, and it does not cover all the mental phenomena observed after LSD. It had the advantage that a systematic record of certain symptoms was obtained at definite intervals before and after administration of the drug. The data obtained in the same group of subjects are reproducible and good dose-effect responses are obtained after different doses of LSD (ISBELL et al. 1956).

[3] The clinical grade was based on the mental status examination, and grades were assigned according to the following scheme: Grade 0, no reaction; Grade 1, anxiety and nervousness without perceptual distortion or hallucinations; Grade 2, anxiety, nervousness, and visual perceptual distortion without "true" hallucinations; Grade 3, anxiety, nervousness, perceptual distortion and "true" hallucinations, but with insight maintained (patients report that effects are due to

Experimental designs. Three separate experiments were done:

Experiment 1. Simultaneous administration of BOL-148 and LSD-25. Fifteen subjects were used in these experiments. Three received 2 mg of BOL-148 plus 0.5 mcg of LSD-25, 3 received 2 mg of BOL-148 and 1 mcg/kg of LSD-25, 3 received 4 mg of BOL-148 and 1 mcg/kg of LSD-25, 3 received 2 mg of BOL-148 and 1.5 mcg/kg of LSD-25, and 3 received 4 mg of BOL-148 plus 1.5 mcg/kg of LSD-25. On another occasion, all subjects received the same doses of LSD-25 but were given no BOL-148. The tests were done at intervals of at least one week in order to prevent the development of tolerance. The order of administration of LSD-25 with and without BOL-148 was randomized. The variations in the doses of BOL-148 and LSD-25 were used to obtain preliminary information on dose-effect relationships which might occur.

Experiment 2. Pre-treatment with BOL-148 for two days. Eight subjects were used in this experiment. These patients received 1 mg of BOL-148 at 8 a.m., 4 p.m. and 10 p.m. for two days prior to being "challenged" with LSD-25. On the morning of the third day they received an additional milligram of BOL-148 at 6 a.m. At 8 a.m. on the test day they were challenged with 1 mcg/kg of LSD-25. On another occasion, all these patients received BOL placebo three times daily for two days and at 6 a.m. on the test day, after which they were "challenged" at 8 a.m. with 1 mcg/kg of LSD. The order of pre-treatment with BOL and placebo was randomized. Neither subjects nor observers were aware of what medications were being given.

Experiment 3. Pre-treatment with 3 mg of BOL-148 daily for five days. Ten subjects were used in this experiment. It was carried out in exactly the same manner as Experiment 2, except that the patients received 1 mg of BOL-148 three times daily for five days plus an additional milligram at 6 a.m. on the day of "challenge" with LSD. There was, of course, a similar experiment in which BOL placebo was given with the order of administration of BOL and placebo being randomized among the subjects.

Analysis of Data

In all experiments the two pre-drug measurements of blood pressure and pupillary size prior to administration of BOL were averaged. The value obtained on each of these measurements after administration of LSD was subtracted from the pre-drug mean. The area under the

drugs), and Grade 4, same as Grade 3, except that insight (realization that the effects are due to the drug) is lost. The grading system has the disadvantage that the various grades may not form a continuous scale. It gives no information concerning the quantitative aspects of the symptoms which go into determining the grades. Like the questionnaire, however, it yields reproducible data on repeated administration of the same dose of LSD, and good dose-effect responses are obtained.

time-action curve composed of these values was calculated by the method of WINTER and FLATAKER (1950), thus converting all the data for a particular experiment, a particular subject, and a particular day to one figure. The number of positive responses on the questionnaire was counted for the entire period after administration of LSD, eliminating positive responses which were also scored positively before the drug. Means and standard errors were calculated by standard statistical techniques (EDWARDS 1946). Differences in the responses on blood pressure, pupillary size, questions, and grade after pre-treatment with BOL-148 as compared with pre-treatment with placebo were evaluated by the t-test for paired observations (EDWARDS 1946). Differences in the number of positive responses on the questionnaire and clinical grade were also evaluated by a nonparametric test (WILCOXON 1949).

Results

Experiment 1. There were no significant differences between any of the measurements when BOL was given simultaneously with LSD as compared with the measurements when LSD was given alone. The lack of any significant change was apparent at all dose levels with LSD and with both doses of BOL. The data for all dose combinations were accordingly combined and are presented in Table 1.

Table 1. *Effect of simultaneous administration of 2—4 mg of BOL-148 on the reaction induced by 0.5 to 1.5 mcg/kg of LSD-25*

Measure[1]	LSD-25 + BOL Placebo (A)	LSD-25 + BOL-148 (B)	Difference[2] (B—A)
Blood pressure[3] . . .	+115.2± 0.31	+132.8 ±11.48	−17.6 ±20.5
Pupillary size[3] . . .	+ 19.7± 1.34	+ 19.58± 1.58	+ 0.12± 1.33
Number of positive responses on questionnaire[4]	60.8±11.3	68.1 ±14.3	+ 7.3 ±10.5
Clinical grade[5] . . .	1.9± 0.3	1.5± 0.24	− 0.4 ± 0.25

Figures are the means ± standard errors of observation on 15 subjects. For details of dosage, see text.

[1] For methods of measurement, see text.

[2] Calculated by a method for paired observations. None of the differences are significant. The signs indicate a decrease (—) or an increase (+) in the measurement after pre-treatment with BOL-148 as compared with placebo.

[3] Figures are means ± standard errors of areas under time-action curves ("mm-hours"); except in the difference column the signs indicate increases (+) or decreases (—) in the measurement.

[4] Means ± standard errors of number of questions scored positively in the $7^1/_2$ hours after the drug, which were not also scored positively before the drug.

[5] Based on scale of 0—4.

Experiment 2. In Experiment 2, a tendency to a reduction in all measurements was observed after pre-treatment with BOL as compared with the responses observed after pre-treatment with BOL placebo. The differences however, while strongly suggestive, did not reach statistically significant levels. The data are presented in Table 2.

Table 2. *Response to 1 mcg/kg of LSD-25 after 3 mg of BOL-148 for two days, as compared with the response after two days of placebos*

Measure[1]	LSD-25 + BOL Placebo (A)	LSD-25 + BOL-148 (B)	Difference[2] (B—A)
Blood pressure[3] . . .	+109.3 ± 12.2	+77.4 ± 16.7	−31.9 ± 14.3
Pupillary size[3] . . .	+ 18.5 ± 2.12	+14.6 ± 1.56	− 3.9 ± 2.55
Number of positive responses on questionnaires[3]	92.5 ± 24.9	53.1 ± 17.6	−39.4 ± 16.7
Clinical grade[3] . . .	2.9 ± 0.3	2.0 ± 0.4	− 0.9 ± 0.55

Figures are the means ± standard errors of observations on 8 subjects.

[1] For method of measurement, see text.

[2] Calculated by a method for paired observations. None of the differences are significant. The negative (—) signs indicate the measurement was reduced after pre-treatment with BOL-148 as compared with pre-treatment by a placebo.

[3] See Table 1 for explanations.

Experiment 3. In this experiment definite attenuation in all the aspects of the LSD reaction measured was apparent and all the differences were either highly significant ($P < 0.01$) or significant ($P < 0.05$). The data are presented in Table 3.

Table 3. *Response to 1 mcg/kg of LSD-25 after 3 mg of BOL-148 daily for five days, as compared with response after five days of placebo*

Measure[1]	LSD-25 + BOL Placebo (A)	LSD-25 + BOL-148 (B)	Difference[2] (B—A)
Blood pressure[3] . . .	+99.9 ± 17.1	+50.3 ± 11.8	−49.6[4] ± 17.8
Pupillary size[3] . . .	+16.0 ± 1.78	+ 9.6 ± 1.65	− 6.4[5] ± 1.88
Number of positive responses on questionnaire[3]	65.9 ± 13.4	27.9 ± 13.6	−38[5] ± 10.7
Clinical grade[3] . . .	2.0 ± 0.36	0.9 ± 0.45	− 1.1[4] ± 0.45

Figures are the means ± standard errors of observations on 10 subjects.

[1] See text for methods of measurement.

[2] Calculated by method for paired observations. The negative (—) signs indicate that the measurement was reduced after pre-treatment with BOL as compared with pre-treatment with placebo.

[3] See Table 1 for explanations.

[4] Significant $P < 0.05$.

[5] Highly significant $P < 0.01$.

Discussion

Results of the three experiments are very clear. In the doses used, BOL administered simultaneously with LSD had no significant effect on the LSD reaction. When BOL was administered in repeated doses for two days prior to administration of LSD, suggestive but not significant reductions were observed in all aspects of the reaction. Administration of BOL-148 for five days reduced both "objective" (blood pressure and pupillary size) and "subjective" (questions and clinical grade) measures to a significant degree. There is obviously a correlation between the length of the period of chronic administration of BOL and the degree of attenuation of the LSD reaction.

Since simultaneous administration of BOL-148 with LSD-25 does not produce any definite attenuation of the LSD-reaction, it seems unlikely that the decrease in the effects of LSD-25 after several days pre-treatment with BOL-148 is due to simple molecular competition between the drugs for receptor sites. The fact that the degree of attenuation of the actions of LSD-25 increases with an increase in the time of chronic pre-treatment with BOL-148 is more compatible with the development of cellular tolerance due to some adaptive change in the physiological or biochemical activity of neurones which requires some time to develop.

Since some authors have postulated that LSD causes a psychosis by blocking the actions of serotonin in the central nervous system, and since both LSD-25 and BOL-148 are serotonin antagonists in isolated preparations of smooth muscle, one might also postulate that cross tolerance between BOL-148 and LSD-25 was due to some adaptive change involving serotonin actions or metabolism within the central nervous system. This possibility, however, seems unlikely. The attenuation of the LSD-reaction observed after pre-treatment with BOL-148 is far less than that which occurs after pre-treatment with LSD despite the fact that BOL is as potent as LSD as a serotonin antagonist and despite the fact that the daily doses of BOL-148 were 20 to 30 times greater than those used in developing tolerance to LSD-25 (Isbell *et al.* 1956). Moreover, Abramson *et al.* (1958) found that, although a high degree of cross tolerance to LSD can be produced by pre-treatment with D-1-methyl-lysergic acid diethylamide (MLD-41), which is nearly three times as potent as LSD as a serotonin antagonist but only one-third as potent as a psychotomimetic, larger doses of MLD-41 are required than was the case with LSD. In addition, we have found, in unpublished experiments, that a degree of cross tolerance to LSD comparable to that described in this experiment can be developed by pre-treatment with 0.5—1.0 mg of D-lysergic acid monoethylamide (LAE-32) daily for seven days. LAE-32 is approximately $^1/_{20}$th as

potent as LSD as a psychotomimetic (ISBELL *et al.* 1959) and $^1/_{10}$th as potent as a serotonin blocker. Therefore, no clear correlation exists between the development of cross tolerance and potency in blocking the action of serotonin on isolated smooth muscle preparations.

The data, however, do suggest that the ability of MLD-41, LAE-32, and BOL-148 may be correlated with their relative potencies as psychotomimetics. More critical tests of this hypothesis might be gained by studying cross tolerance in congeners of LSD-25 that are even more inert than BOL-148. Examples of such substances would be 1-methyl-2-brom-D-lysergic acid diethylamide (MBL-61) and 1-methyl-D-lysergic acid butanolamide (UML-491).

BALESTRIERI (1957) has recently reported that pre-treatment with LSD-25 for three to five days in doses increasing to 200—250 mcg daily reduced the reaction caused by 0.5 grams of mescaline intravenously. Such a result suggests that development of cross tolerance may be more dependent on similarity of pharmacological effect than on similarity of chemical structure.

Summary

1. Simultaneous administration of 2 to 4 mg of D-2-brom lysergic acid diethylamide (BOL-148) did not reduce the intensity of the reaction caused by 0.5 to 1.5 mcg/kg of LSD-25.

2. Pre-treatment of 8 subjects with 3 mg of BOL-148 daily for two days caused statistically nonsignificant reductions in all aspects of the reaction induced by 1 mcg/kg of LSD-25.

3. Pre-treatment of 10 subjects with 3 mg of BOL-148 for five days resulted in statistically significant attenuation of all aspects of the LSD reaction that were measured.

4. The results are more compatible with the development of cross tolerance between BOL-148 and LSD-25 than they are with simple molecular competition between the two drugs.

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