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LYSERGIC ACID DIETHYLAMIDE (LSD-25): XXIII. COMPARATIVE EFFECTS OF LSD-25 AND RELATED ERGOT DRUGS ON BRAIN TISSUE RESPIRATION AND ON HUMAN BEHAVIOR*

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A. INTRODUCTION

In a search for a possible biochemical mechanism by which d-lysergic acid diethylamide (LSD) causes schizophrenia-like symptoms in man, it has been found that the respiration of crude guinea pig brain preparations is affected by this drug. Thus, Clark, Fox, Benington, and Morin (4) have reported that 10^{-3} M LSD-25 (a soluble LSD tartrate) increases cytochrome *C* oxidase activity and decreases succinoxidase activity in guinea pig brain homogenates. Lewis and McIlwain (8), working with electrically stimulated slices and minces, have demonstrated an inhibition of respiration by concentrations of LSD of the order of magnitude of 10^{-5} M. Although both groups of investigators have reported data with one or two ergot drugs other than LSD, neither group has adduced data concerned with clarifying the differences between LSD-25 and other ergot derivatives on brain tissue and on man himself. A study was therefore undertaken to compare the effects of LSD-25 with that of eight other ergot derivatives upon the oxygen consumption of readily prepared guinea pig brain preparations and to ascertain whether a correlation exists between *in vitro* activity of ergot derivatives and their psychopharmacologic effect upon man. The psychological effects upon man of related drugs (2, 7) are reported elsewhere. It was necessary, however, for us to investigate the action of l-LSD-25 upon human volunteers.

B. METHODS AND MATERIALS

A 400-g guinea pig was killed by a sharp blow across the back. Its cerebrum was removed and washed in 3 ml of buffer solution. The tissue was

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then either homogenized or minced with a razor blade. When an homogenate was prepared, the washed and weighed brain was ground with about twice its weight of buffer in a Potter homogenizer and then further diluted. Aliquots of the homogenate were pipetted into Warburg vessels whose sidearms contained appropriate test solutions and whose center wells contained 0.2 ml of 2N NaOH. The vessels were equilibrated for at least 15 minutes before the manometers were closed, zero time readings taken, and the sidearm contents tipped into the tissue mixture. Readings in the experiments were begun 50 to 60 minutes after the death of the guinea pig, by which time a relatively steady rate of O_2 consumption had supervened. Speed was important only in the period from the death of the animal to the beginning of homogenation. The experiments with homogenates were done in duplicate.

The minced tissues were washed by suspending in 5 ml of buffer and filtering with suction. Portions of the mince weighing about 200 mg were transferred to Warburg vessels, to each of which was then added 2.7 ml of buffer. Alkali was added to the center wells, and 0.2 ml quantities of the appropriate solutions were added to the sidearms. The vessels were shaken in the Warburg bath for 45 minutes before readings were begun to make certain that the tissue had settled down to a steady rate of oxygen consumption. This rate generally changed little in the next three hours. Because the weighing of samples of mince was rather inaccurate, the following technique, which did not require a precise knowledge of the amount of tissue in each flask, was employed: Readings were taken for 75 minutes to establish the rate of oxygen consumption for each flask in the absence of drug. The manometers were then opened, reset and closed again. The new zero readings were taken and the contents of the sidearms tipped into the vessels. Readings were taken at 15-minute intervals and the oxygen consumption of each drug-containing vessel was compared to the oxygen consumption of the control vessel by means of this formula:

$$\text{Relative } O_2 \text{ Consumption} = \frac{\text{UPTAKE OF TEST VESSEL AFTER TIPPING}}{\text{UPTAKE OF CONTROL VESSEL AFTER TIPPING}} \times \frac{\text{UPTAKE OF CONTROL VESSEL BEFORE TIPPING}}{\text{UPTAKE OF TEST VESSEL BEFORE TIPPING}}$$

In all the metabolic experiments reported in this paper, preparations were made at room temperature and Warburg studies were made at 37° C with air as the gas phase. As long as the total oxygen consumption per vessel was considerably over 100 microliters (as was the case in experiments last-

ing 75 minutes) the difference between duplicate vessels was less than 5 per cent. The mode of graphic presentation of data that has been used below, based as it is upon relatively short time intervals, may at times suggest a greater degree of uncertainty in the results than is indeed the case.

The method of assaying the l-LSD-25 on man was described in detail previously (2). Briefly, the technique employs non-psychotic subjects who form the basis of a test group. These subjects are used over a period of years and are paid $20 per test. They are subjects who have shown by repeated trials that they can readily detect approximately 25 gamma of d-LSD-25 orally. A questionnaire consisting of two sections is used. The first section contains 47 items which list the physiological and perceptual symptoms reported in the literature. Only a positive response indicates the presence of a given symptom. The second part of the questionnaire consists of the eight items listed in Figure 5, i.e., motor behavior, control, consciousness, concentration, mood, attitude, orientation, and memory. In this series of experiments observations were made on three test subjects for at least three and one-half hours. The number of positive responses to the first section of the questionnaire are totaled as indicated in Figure 5. The sum of these numbers plus the subjects' and observer's evaluation of the eight items in section two of the questionnaire are compared with placebo experiments, which are repeatedly used to check the validity of the test subjects' evaluation of the presence of a drug in a given experiment.

The ergot derivatives used in these experiments included d-lysergic acid, d-lysergic acid diethylamide tartrate (LSD-25), l-lysergic acid diethylamide tartrate (l-LSD-25), d-isolysergic acid maleate (d-isoLSD-25), d-lysergic acid monoethylamide (LAE-32), BOL-148 (LSD-25 brominated at the indol ring), ergonovine tartrate, ergotamine tartrate, and dihydroergotamine methanesulfonate. They were employed as water solutions except for d-lysergic acid which was dissolved in dilute NaOH (final pH = 7.8) and ergotamine and d-isoLSD-25 which were dissolved in 10^{-3} M HCl.

The buffer used in the experiments being reported was a sodium Ringer phosphate buffer such as described by Cohen (5) but lacking Ca^{++} and containing 2.2×10^{-3} M glucose and 4.4×10^{-3} M dl-lactate. When this buffer was adjusted with NaOH to pH 7.6 before use, the final pH was about 7.4 in the mince-containing flasks and about 7.2 in the homogenate-containing flasks. The use of a mixture of glucose and lactate (both of which are suitable substrates for LSD-25 studies) supplemented the phosphate in buffering action; for, when lactate was the sole substrate, the pH rose to values of 7.6 and higher, and, when only glucose was added, the final

pH was in the vicinity of 6.8. Substitution of potassium for sodium did not in any way affect the results obtained with homogenates. The addition of Ca^{++} decreased both the total oxygen consumption and the magnitude of the inhibition.

C. Experimental Results

1. *Homogenates*

Figure 1 shows the results of two typical experiments with guinea pig brain homogenates at a concentration of 2.5×10^{-4} M of the various ergot

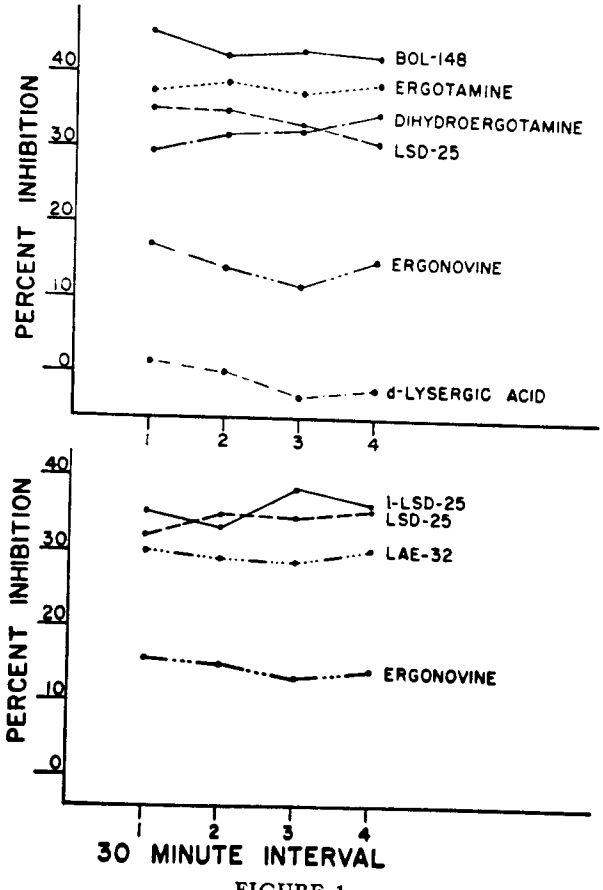

FIGURE 1

The effect of 2.5×10^{-4} M solutions of ergot derivatives upon respiration of homogenized guinea pig brain. Top: 156 mg tissue/vessel; QO_2 (wet weight basis) of control = 4.3 for first hour; 3.5 for second hour. Bottom: 193 mg tissue/vessel; QO_2 of control = 3.6 for first hour; 3.1 for second hour.

derivatives. Only d-lysergic acid is completely inactive at this concentration while l-LSD-25, ergotamine, dihydroergotamine, and BOL-148 are at least as active as LSD-25 itself. Experiments at the 2.5×10^{-4} M level are not reported for d-isoLSD-25 because of the uncertainty resulting from the insolubility of this compound. However, d-isoLSD-25 is at least as inhibitory as LAE-32.

Because LSD-25 is physiologically active in man at concentrations of the order of 10^{-9} M, assuming uniform distribution through the tissues, attempts were made to repeat the homogenate experiments at lower concentrations of ergot drugs. It was soon found that the concentration of LSD-25 necessary to bring about a significant inhibition of oxygen consumption was a function of the concentration of tissue in the homogenate. Table 1 shows the effect of varying both concentration of tissue and LSD-25. From these data it is clear that the effect of LSD-25 upon guinea pig brain homogenates depends upon the ratio of drug concentration to tissue concentration. It should, therefore, be possible to achieve inhibition with very low concentra-

TABLE I

VARIATION OF INHIBITION OF OXYGEN CONSUMPTION WITH THE RATIO OF LSD-25 CONCENTRATION TO TISSUE CONCENTRATION (Guinea Pig Brain Homogenates)

a.	63 mg brain tissue per ml			21 mg brain tissue per ml	
Concentration of LSD-25 10^{-5} Molar	0	4	4/3	0	4/3
Conc. LSD-25 / Conc. Tissue (Moles 10^{-10} / mg)	—	6.3	2.1	—	6.3
Uptake in 145 min.	281	233.5	260	103	82.5
Inhibition per cent	—	16.8	7.4	—	20

b.	57 mg brain tissue per ml			19 mg brain tissue per ml	
Concentration of LSD-25 10^{-5} Molar	0	4	2	0	0.67
Conc. LSD-25 / Conc. Tissue (Moles 10^{-10} / mg)	—	7.0	3.5	—	3.5
Uptake in 165 min.	278	226.5	246	96.5	86.5
Inhibition per cent	—	18.5	11.5	—	10.5

tions of the drug by diminishing the amount of tissue employed, providing (*a*) the mass ratio relationship holds, (*b*) the QO_2 of the tissue does not decrease with dilution, and (*c*) the technique being employed is sufficiently sensitive. Since the percentage error in a Warburg experiment increases with the decrease in rate of O_2 consumption, the technique was self-limiting, and it was decided to maintain the tissue level at between 100 and 200 mg per vessel (total volume = 3.0 ml) even for the experiments with a lower concentration of ergot derivatives. It was, therefore, difficult to get significant and reproducible inhibition with less than 4×10^{-5} M LSD-25. The data in Figure 2 show that in experiments at this lower level the relative activity of the various ergot derivatives stay about the same.

It is interesting to note, at this point, that an appreciable quantity (possibly all) of the LSD-25 used in these experiments can be recovered when, after an experiment like that in Figure 2, the contents of the vessels are removed and allowed to settle overnight in the refrigerator. The supernatant liquid from the LSD-25 vessel shows no loss in activity detectable by the Siamese fighting fish titration of Abramson and Evans (1) when compared with an appropriate dilution of fresh LSD-25.

2. *Minces*

It is generally agreed that the physiological action unique to a given drug may lie not only in the biochemical properties of the drug but also in the drug's ability to penetrate to a strategic site not readily reached by even closely related compounds. By mincing the brain instead of completely destroying its cells and their membranes, it is possible to preserve what may be a most important element in the blood-brain barrier. Figures 3 and 4 show the results of experiments with such preparations at the two drug levels used in the experiments with homogenates. LSD-25 and its isomers and closely related derivatives seem to exert their effect as if the cell membranes offered no barrier. Ergotamine and dihydroergotamine are relatively inactive at the lower concentration used, and show an induction period before exerting their full effect at the higher concentration. This increase of effectiveness with time has also been observed in the case of dihydroergotamine by Lewis and McIlwain (8).

Figure 5 is an example of a spectacular difference in the reaction of the l-isomer and the d-isomer. Subject L.W. was particularly chosen not only because he was able to detect small quantities of LSD-25, but over a period of a year he invariably could detect a placebo. One hundred micrograms of the l-isomer was the highest dose that has been administered to our three

test subjects. It is conceivable that some LSD-25 activity is present, but it is certainly less than 15 per cent of the d-isomer. The fourth section of Figure 5 illustrates that the administration of 100 micrograms of the l-isomer gave no protection against 25 micrograms of LSD-25 within the limits of error of our method.

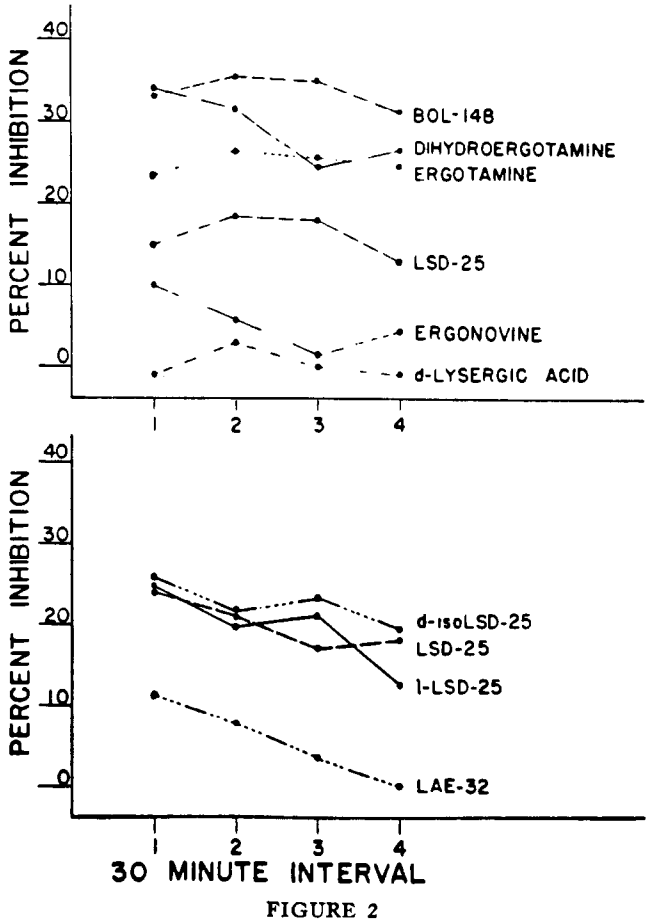

FIGURE 2

The effect of 4×10^{-5} M solutions of ergot derivatives upon respiration of homogenized guinea pig brain. Top: 103 mg tissue/vessel; QO_2 (wet weight basis) of control = 3.5 for first hour; 3.3 for second hour. Bottom: 187 mg tissue/vessel; QO_2 of control = 3.3 for first hour; 3.0 for second hour.

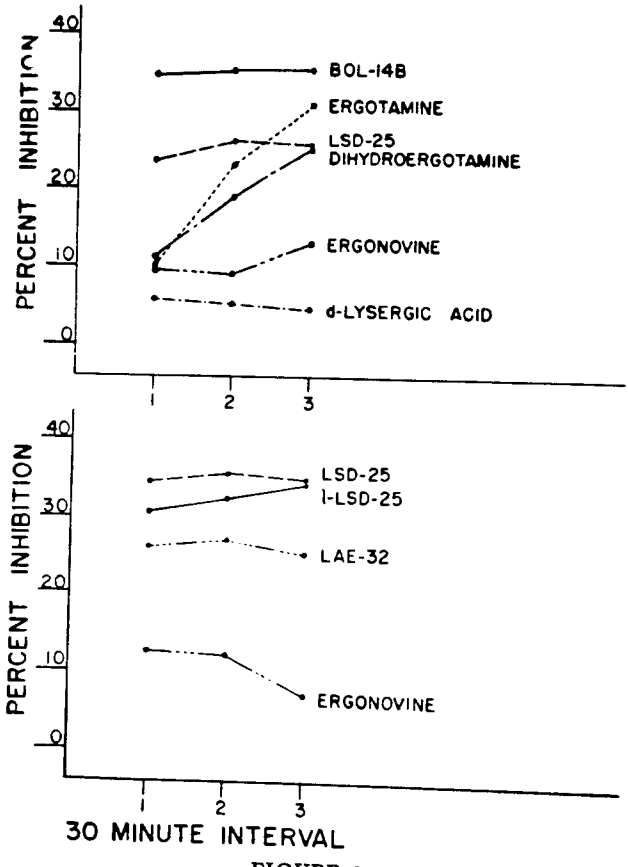

FIGURE 3

The effect of 2.5×10^{-4} M solutions of ergot derivatives upon respiration of minced guinea pig brain. Top: 225 mg tissue in control vessel; QO_2 of control = 3.8 for first hour; 3.6 for second hour. Bottom: 210 mg tissue in control vessel; QO_2 of control = 4.8 for first hour; 4.6 for second hour.

D. Discussion

One of the properties of the effect of LSD-25 on man is the specificity of the molecule involved in the reaction. Thus, ergotamine, dihydroergotamine, and ergonovine at relatively high dosages have been used in medicine without marked psychologic effects. Moreover, Jarvik, Abramson, and Hirsch (7) have shown in carefully controlled experiments that the effect of 1 microgram/kilogram of body weight of LSD-25 is greater than the effect of 5 to 7 micrograms/kilogram of body weight of either LAE-32 or

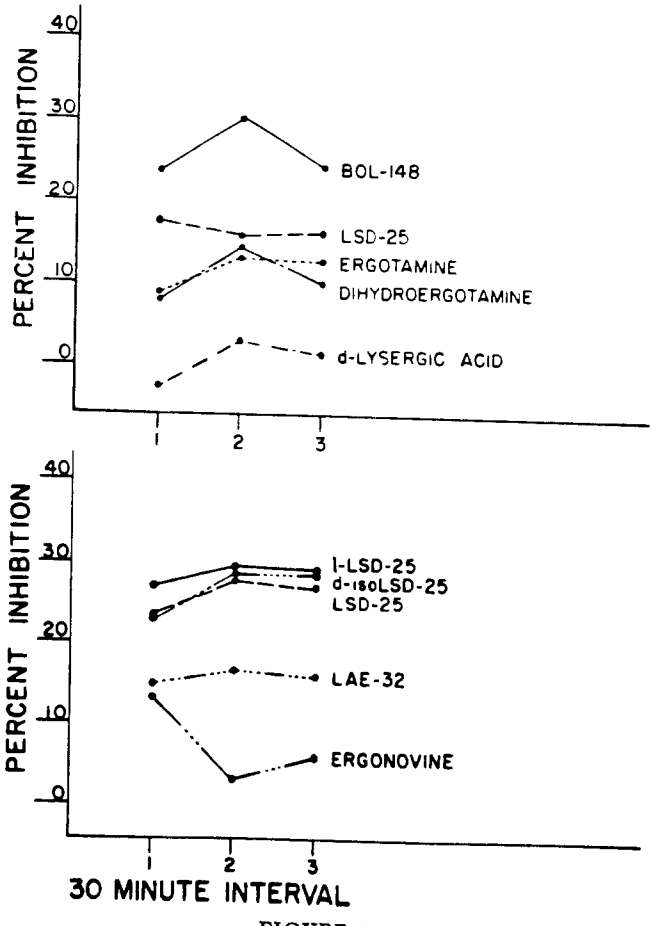

FIGURE 4

The effect of 4×10^{-5} M solutions of ergot derivatives upon respiration of minced guinea pig brain. Top: 180 mg tissue in control vessel; QO_2 of control = 4.7 for first hour; 4.7 for second hour. Bottom: 198 mg tissue in control vessel; QO_2 of control vessel = 4.9 for first hour and 4.7 for second hour.

BOL-148. Similar results with BOL-148 have been reported by Cerletti and Rothlin (3). The effect of 650 micrograms per subject ($\cong$ 10 micrograms/kilogram) of ergonovine was even less than that of the BOL-148 (which, in turn, was less like LSD-25 than was LAE-32). Finally, in our own experiments on man, no response to 100 microgram doses of l-LSD-25 was observed in subjects who reacted strongly to 25 microgram doses of LSD-25.

HOURS AFTER INGESTION OF DRUG

		½	1½	2½	3½	4½	later
Subj. L. W. Date 6/19/54	#Positive responses	4	8	9	8		
	Motor behavior		poor	poor	poor		
	Control	poor	poor	poor			
LSD-25	Consciousness	poor	poor	poor			
25 micrograms	Concentration	poor	poor				
	Mood	euph.	euph.	euph.			
	Attitude						
	Orientation						
	Memory						
	Remarks	Conscious of people pronouncing words.					

Subj. L. W. Date 9/17/54	#Positive responses						
	Motor behavior						
	Control			N O			
L-isomer	Consciousness						
25 micrograms	Concentration						
	Mood						
	Attitude		R E	A C	T I O	N	
	Orientation						
	Memory						
	Remarks						

Subj. L. W. Date 9/24/54	#Positive responses						
	Motor behavior						
	Control			N O			
L-isomer	Consciousness						
100 micrograms	Concentration						
	Mood						
	Attitude		R E	A C	T I	O N	
	Orientation						
	Memory						
	Remarks						

Subj. L. W. Date 10/1/54	#Positive responses	2	7	9	6		
	Motor behavior		poor	poor	poor		
	Control		poor	poor	poor		
LSD-25	Consciousness						
25 micrograms	Concentration		conf.	conf.	conf.		
	Mood	depr.	euph.	euph.	euph.		
L-isomer	Attitude		intro.	intro.	intro.		
100 micrograms	Orientation		poor	poor	poor		
1/4 hr. before	Memory						
LSD-25	Remarks						

FIGURE 5

Summary of psychodynamic effects upon man at LSD-25 and l-LSD-25 when administered singly and together.

The Siamese fighting fish test for LSD-25 is also remarkably specific. The early stages of BOL-148 intoxication and the stupor induced by relatively large dosages of LAE-32 resemble in some respects the effect produced by as little as 0.2 micrograms/milliliter of LSD-25. However, none of the other ergot derivatives that we have tested produce a response similar to that of LSD-25 even when a molar concentration equivalent to that of 20 micrograms/milliliter of LSD-25 is used [Evans, Geronimus, Kornetsky, and Abramson (6)].

In contrast to the special effects of LSD-25, LAE-32, and BOL-148 on man and on fish, partial inhibition of the over-all oxygen consumption of crude guinea pig brain preparations is brought about, to a greater or lesser degree, by eight of the nine ergot derivatives tested. This would make it seem that the reactions involved in the *in vitro* effects have no relationship to the *in vivo* effects of LSD-25. However, a closer inspection of the data shows that it is still possible that some of the observations that have been made in this study may involve mechanisms operative in the physiological effect of LSD-25.

It is evident from the experiments on man and on the Siamese fighting fish that the peculiar activity of LSD-25 is a resultant of at least two of its properties: (*a*) the configuration of the d-lysergic acid nucleus, and (*b*) the nature of the side-chain. Thus, only derivatives of unsubstituted dextro lysergic acid are active and, within the d-series of compounds, the diethylamide has the highest activity, followed in order of decreasing activity by the ethyl amide, the isopropyl alcohol amide and, at least in the case of the Siamese fighting fish, unsubstituted d-lysergic acid. This is precisely the order of activity shown by these compounds as inhibitors of guinea pig brain respiration *in vitro*. Thus, by applying separately these two criteria mentioned above it can be seen that the manometric experiments, although they do not discriminate between compounds differing in the configuration of their lysergic acid nuclei, may serve to differentiate between derivatives of unsubstituted d-lysergic acid.

Ergotamine and dihydroergotamine are d-lysergic acid derivatives which have a strong effect upon the respiration of cell-free brain preparations but relatively low psychodynamic effects. These results can still be reconciled with the scheme laid out above on the basis of the difficulty with which they enter reasonably intact tissue. It may be noted here parenthetically that the activity shown by these drugs under the experimental conditions of Figure 4a was generally lower. The set of data was chosen primarily because it included, besides ergotamine and dihydroergotamine, the other ergot derivatives required to make the figure complete.

E. Summary

A technique is described for the preparation of guinea pig brain homogenates and minces whose respiration is susceptible to low concentrations of d-lysergic acid diethylamide (LSD-25) even in the absence of electrical stimulation. The effects of LSD-25 and of other ergot derivatives upon these preparations are compared. Ergotamine, dihydroergotamine, 2-bromo d-lysergic acid diethylamide (BOL-148), l-LSD-25, d-isoLSD-25, d-lysergic acid ethylamide (LAE-32), and ergonovine, as well as LSD-25, cause a greater or lesser diminution of oxygen consumption, with a mixture of glucose and lactate as substrate. Only d-lysergic acid, of the nine compounds tested, is completely without effect. All the inhibitory compounds, except ergotamine and dihydroergotamine exert their inhibition upon minces as well as homogenates immediately upon addition to the tissue preparations. Ergotamine and its dihydro derivative, however, require an induction period before exerting their full inhibitory effect upon minces in a concentration of 2.5×10^{-4} M and are relatively inactive in a concentration of 4×10^{-5} M. Among the diethylamides the optical isomers of LSD-25 are just as inhibitory as is LSD-25 itself; the brominated derivative is more inhibitory. This contrasts sharply with the effects upon man. BOL-148 shows a low degree of psychodynamic activity, while l-LSD-25 is inactive up to 100 micrograms by mouth.

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