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AN EXPERIMENTAL STUDY OF THE PARTICIPATION OF THE SYMPATHETIC NERVOUS SYSTEM IN THE LYSERGIC ACID DIETHYLAMIDE (LSD) REACTION IN CATS[1]

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Among the first investigations into the mechanism of action of LSD were those concerned with its antagonism to serotonin (Gaddum, 1953; Gaddum and Hameed, 1954). These consisted primarily of studies upon isolated tissue and hence their results were difficult of application to possible drug interactions in the central nervous system. A more serious obstacle to the acceptance of LSD-serotonin antagonism as the mechanism of action of LSD is presented by the lack of psychotomimetic potency of the 2-Brom derivative of LSD (BOL) which is at least equipotent as a serotonin antagonist (Cerletti and Rothlin, 1955). Further doubt of the significance of this phenomenon as a mechanism is created by the fact that rather than showing antagonistic actions throughout, LSD and serotonin in some cases have been shown to produce the same qualitative effects (Horita and Gogerty, 1958; Brodie and Shore, 1957), and BOL has been shown to antagonize the effects of both LSD and serotonin (Horita and Gogerty, 1958).

One of the most consistent findings in animals has been evidence of sympathetic discharge (Rothlin *et al.*, 1956; Bogdanski and Spector, 1957). Perhaps the most striking demonstration of this is seen in the LSD-induced "rage" reaction in cats (Gaddum and Vogt, 1956). Bard (1928) indicated the necessity of an intact posterior hypothalamus for the production of decorticate rage in cats, a fact which ties in with what apparently is happening in LSD-treated animals; *i.e.*, sympathetic discharge accompanies this rage phenomenon whether induced surgically or pharmacologically. Several workers have indicated that catecholamine levels are higher in schizophrenics and subjects treated with psychotomimetics than in normals (Liddell and Weil-Malherbe, 1953; Sulkowitch *et al.*, 1957; Manger *et al.*, 1957). Hence, the overall picture of LSD symptomatology appears to be associated with excessive sympathetic discharge.

This study was undertaken to determine the role of the sympathetic nervous system and its neurohumors in the production of the LSD-induced rage reaction in cats. Phenoxybenzamine (Dibenzyline) and hydralazine were used as adrenergic blocking agents, the former as an example of the agents used to produce peripheral adrenergic blockade and the latter as an example of an adrenergic blocking agent with a component of central activity, although the site of action of this compound is disputed (Walker *et al.*, 1951; Bein, 1953). Total surgical sympathectomy was also performed in order to ascertain whether any antagonistic effect of the adrenergic blocking agents used was due to the peripheral blockade so induced or to a central effect of these drugs. Atropine and scopolamine were used as classical anticholinergic agents in order to determine the effects of parasympathetic blockade on this reaction. Hexamethonium was used as a means of causing blockade of both parasympathetic and sympathetic systems. Reserpine and chlorpromazine were used as examples of tranquilizing agents which alter the manifestations of sympathetic discharge.

METHODS. *Evaluation of the LSD reaction.* Adult male and female cats received LSD, 400 μg/kg *via* the saphenous vein, and the behavioral changes so induced were evaluated on a protocol sheet such as that in table 1. Each component of the reaction was rated on an arbitrary scale of 0 to 3, in which 0 indicated the absence of that component and 3 represented the most intense reaction. Only those animals demonstrating a well-sustained and well-directed aggression were

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Salivation
Piloerecti
Blindness

SPONTANEOU
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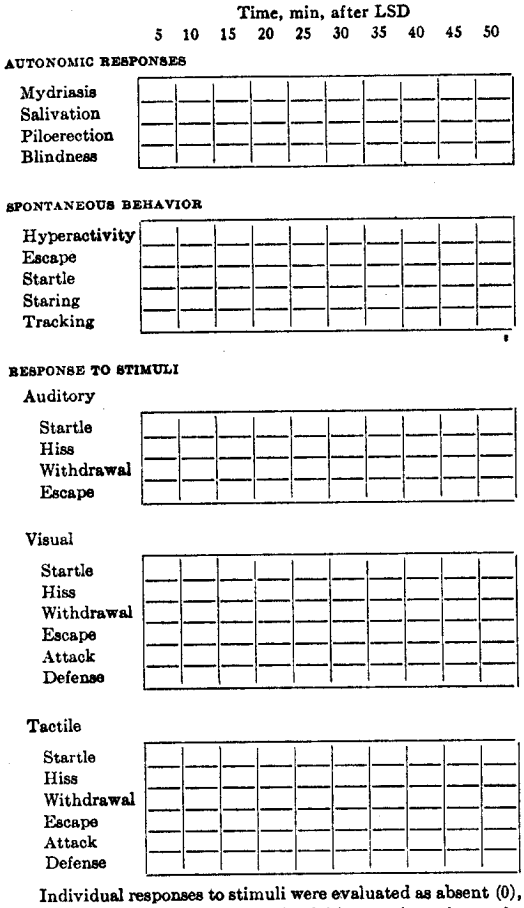
TABLE 1

Evaluation protocol of the LSD response in cats



Individual responses to stimuli were evaluated as absent (0), minimal (1), moderate (2), or maximal (3) at 5-minute intervals. Spontaneous behavioral changes were observed and evaluated on the same rating scale during the intervals between stimulus periods.

included in the experimental group. Those animals reacting for less than 25 minutes were also excluded from the study. Auditory stimulation consisted of a sudden loud noise produced by clapping together a hinged board. Tactile and visual stimuli were presented in the forms of an approaching gloved hand or a swinging pendulum consisting of a large rubber stopper suspended from a stout cord. All animals were placed in a wire-enclosed cage with a large plexiglass observation window for the duration of the reaction. In order to quantify the reactions, the numerical values assigned to each response of the particular component of the reaction were averaged and the intensity of the entire reaction was then determined by calculating the mean of the average values for the individual components. Spontaneous behavior was observed in the 5-minute intervals between stimuli. The reaction was considered terminated when the application of stimuli elicited only hissing or a plaintive meowing for at least three successive stimulation periods. The mean scores for the complete reaction as well as the mean scores for the several categories of response were compared with controls by the application of Student's *t* test. After determination of the control LSD response, a period of at least 1 week was allowed to elapse before giving LSD again, because of the onset of tolerance to this material if doses are administered in close sequence (Isbell *et al.*, 1956; Gogerty and Dille, 1956). Control reactions were determined twice in each animal in order to assess the constancy of the response to the several stimuli. Blind evaluation was rendered impossible by the characteristic coloration of some animals, but the schedule of assessments was such that it was unlikely that a previous reaction could be recalled. Two separate evaluations were done in each case. The correlation coefficient for control reactions was 0.81, and for the antagonism series, 0.85.

Surgical procedures. Using the retroperitoneal approach, transplants of the adrenal cortices were made to the subcutaneous tissue of the neck or beneath the renal capsule after removing as much as possible of the medullary tissue. Postoperative care of these animals consisted of 50 ml of 5% dextrose in saline subcutaneously and 25 mg cortisone i.m. daily for 2 days. Survival of the animal after withdrawal of the cortisone was taken as proof of a successful transplant.

Bilateral sympathectomy was performed in two stages using the procedure of Cannon and Rosenblueth (1937) with only slight modification. Postoperatively these animals received 50 ml of 5% dextrose in saline s.c.; 600,000 units of procaine penicillin i.m.; 25 mg of cortisone i.m.; and 10 mg phenylephrine i.m. All but the phenylephrine were repeated daily for 2 days. A recovery period of 7 to 14 days was allowed between stages and before use in these studies. All surgical procedures were performed under ether anesthesia with positive pressure respiration. Only those animals showing full recovery of motor activity and appetite were included in the experimental group.

Drug antagonism studies. The drugs used in attempts to antagonize the LSD response were chlorpromazine HCl,[2] reserpine, atropine SO_4, scopolamine HBr, hexamethonium Cl, hydralazine HCl and phenoxybenzamine HCl.[2] Route of

[2] Kindly supplied by Smith Kline & French Laboratories, Philadelphia, Pennsylvania.

administration and doses for each of these materials will be described in the text. Doses were calculated as the salt of the compound except in the case of reserpine.

RESULTS. *Atropine.* Atropine, 2.0 mg/kg i.m. 60 minutes prior to LSD or 1.0 mg/kg i.v. 5 minutes prior to LSD, produced no significant change in the LSD reaction. The only component of the reaction that was absent consistently was salivation.

Scopolamine. In addition to its anticholinergic activity, this compound is well known for its sedative effect. In doses of 0.2 mg/kg i.m. 60 minutes prior to test or 0.1 mg/kg i.v. 5 minutes before LSD, scopolamine produced some evidence of behavioral sedation during the waiting period before the injection of LSD but did not prevent the full LSD reaction from developing in these animals. As with atropine, salivation was the only component of the response that was absent consistently. These findings with atropine and scopolamine would indicate that there is some difficulty in correlating EEG effects and behavioral effects produced by various drugs (Rinaldi and Himwich, 1955b, c; Wikler, 1952; Himwich *et al.*, 1956; White and Daigneault, 1959). Table 2 summarizes the effects of these compounds.

Hexamethonium. In doses of 10 mg/kg i.v. 10 minutes before the challenge dose of LSD, this drug either attenuated or abolished the autonomic responses to LSD injection but did not affect the behavior of these animals as shown in table 2. The influence of this compound upon the autonomic responses indicated that these responses are of central origin since LSD has no ganglion stimulating effect (Broghammer *et al.*, 1957).

Chlorpromazine. Chlorpromazine, 5.0 mg/kg i.m. daily for 3 days prior to test and an additional dose of the same magnitude 30 minutes before the challenge dose of LSD, obliterated all components of the reaction except the hiss and mydriasis which were reduced to about half their control intensity. As with atropine, chlorpromazine has been shown to prevent the EEG alert pattern in low doses but to cause the appearance of this pattern if given in higher doses (Rinaldi and Himwich, 1955a). In order to determine if this would influence the LSD reaction in a manner different from the 5.0-mg/kg dose, these experiments were repeated using 15 mg/kg of chlorpromazine on the same dosage schedule. During the premedication period these animals were moderately depressed but continued to eat an almost normal amount. The larger dose was approximately as effective as the 5.0-mg/kg dose in preventing the behavioral changes induced by LSD. Table 2 summarizes these results.

Reserpine. Reserpine, 0.5 mg/kg i.m. daily for 6 days prior to receiving LSD, produced pronounced depression of physical activity and appetite. The depression was progressive for about 3 days and then remained at that level. While giving the appearance of severe depression these animals could be aroused by handling and walked about with an ataxic gait if prevented from lying down. Immediately stimulation ceased, however, these animals resumed their previous somnolent attitude. Extreme miosis was present throughout the premedication period.

Administration of the standard dose of LSD produced an almost immediate arousal in these animals and within 60 seconds an LSD reaction of at least control intensity was evident. The pupils of the animals retained the slit-like proportions produced by reserpine. This effect was still present to some degree for 2 days after the test. While the animals essentially were oblivious of all visual stimuli, tactile stimulation produced significantly more intense responses and spontaneous behavioral changes in these animals were more pronounced than in controls. The mean values assigned to these reactions were not significantly different from the controls, but the compensating factor in the overall analysis was the extremely low value assigned to visually elicited responses. For all practical purposes, responses in this category were absent. In all cases, the duration of the reaction was shorter in the reserpine-LSD treated animals than in controls.

About 30 to 60 minutes post-LSD, the animals appeared to be settling into a catatonic-like state and were oblivious of their surroundings and all external stimuli. In order to determine the extent of their oblivion, these cats were placed in awkward, unnatural positions which were maintained until changed by the experimenter or by gravitational force. All cats in this series remained unresponsive to stimuli and position changes for about 24 hours. At no time during this period did an animal exert a voluntary effort to remove itself from a position in which it had been placed.

TABLE 2

Mean ± S.E. of the various categories of response to LSD as influenced by various drugs

Drug	Autonomic	Spontaneous	Auditory	Visual	Tactile	Reaction Mean
Atropine (4)	1.74 ± 0.07	1.38 ± 0.07	1.49 ± 0.06	1.35 ± 0.04	1.54 ± 0.06	1.50 ± 0.06
Scopolamine (4)	1.79 ± 0.10	1.40 ± 0.08	1.45 ± 0.06	1.40 ± 0.08	1.48 ± 0.07	1.50 ± 0.08
Hexamethonium (3)	0.93 ± 0.03*	1.54 ± 0.05	1.65 ± 0.06	1.62 ± 0.04	1.55 ± 0.07	1.45 ± 0.05
Hydralazine (5)	1.80 ± 0.04	1.46 ± 0.03	1.47 ± 0.06	1.49 ± 0.05	1.58 ± 0.07	1.56 ± 0.05
Chlorpromazine† (5)	0.13 ± 0.03	0.09 ± 0.01	0.20 ± 0.04	0.14 ± 0.03	0.19 ± 0.04	0.15 ± 0.03*
Phenoxybenzamine† (4)	0.15 ± 0.06	0.32 ± 0.08	0.30 ± 0.07	0.50 ± 0.08	0.41 ± 0.05	0.33 ± 0.07*
Reserpine (4)	1.79 ± 0.05	2.01 ± 0.06*	1.95 ± 0.08	0.08 ± 0.01*	2.12 ± 0.08*	1.59 ± 0.05
LSD control (30)	1.95 ± 0.10	1.49 ± 0.09	1.51 ± 0.20	1.53 ± 0.14	1.55 ± 0.10	1.61 ± 0.12

Numerals in parentheses indicate the number of animals.
* Values significantly different from control values.
† All categories of response significantly different from control values.

Figure 1 shows the positions in which these animals were placed. About 24 to 36 hours after this condition developed, the attention of the animals could be drawn by tactile or auditory stimuli, but they lapsed into the catatonic-like state immediately the stimulus was withdrawn. Table 2 summarizes the results with reserpine.

Hydralazine. A dose of 10 mg/kg i.v. 15 or 60 minutes before the challenge dose of LSD did not have any great effect upon the LSD reaction. The component of aggression was minimal in all of these animals, which made the reaction appear different from control reactions but statistically no difference could be shown. As indicated in table 2, this compound failed to block the responses attributable to the autonomic nervous system, which fact casts doubt on the efficacy of this drug as an adrenergic blocking agent under the conditions of its use in these experiments.

Phenoxybenzamine. Since hydralazine was ineffective in producing complete peripheral sympathetic blockade, phenoxybenzamine, 15 mg/kg i.v. 30 to 60 minutes before LSD, was used to determine the effect of complete blockade on the LSD reaction. Within 5 minutes of the injection of phenoxybenzamine, the nictitating membranes were relaxed and the cats appeared very calm and tractable. Their tractability was especially noticeable when the animals were restrained for the intravenous injection of the LSD. In these animals, all components of the LSD reaction were reduced markedly in intensity or abolished completely. Although reduced in intensity, tracking, hissing and in some cases the startle usually remained evident. In most instances the aggression component of the reaction was present during the first stimulation period but only rarely could this response be evoked beyond the first 5 minutes of the reaction. When present, the aggressive tendencies of the animals were of minimal to moderate intensity. As shown in figure 2 and table 2, the reactions in these animals were significantly different ($P < .01$) from those elicited in control experiments. In view of the results with hexamethonium, these results are difficult to explain on the basis of a purely peripheral sympathetic blockade.

Adrenal demedullation. Adrenal demedullation and transplantation of the cortices to the subcutaneous tissue of the neck or beneath the renal capsule was carried out as a means of examining the contribution of medullary hormones to the LSD reaction. In animals so treated, LSD produced a reaction comparable to that produced in control experiments. Since it has been shown that residual medullary tissue survives well and retains its ability to elaborate catecholamines in adrenal transplants (Coupland, 1959), a direct effect of LSD on this remaining tissue cannot be discounted completely. However, it is likely that any secretion from this tissue would be minimal because of the relative amount of tissue remaining and the demonstration of the lack of ganglionic stimulation by LSD (Broghammer *et al.*, 1957).

Surgical sympathectomy. Since phenoxybenz-

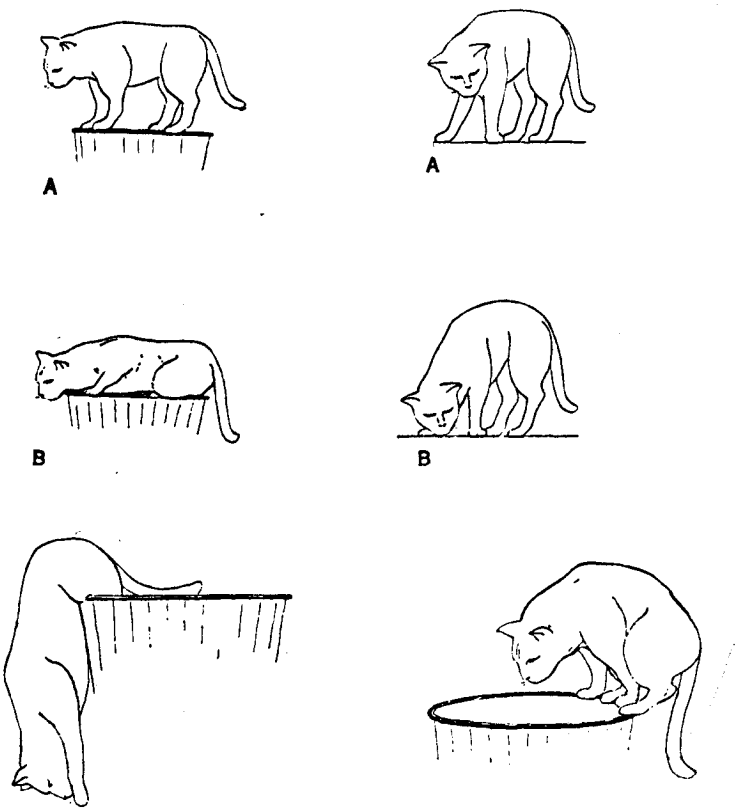


FIG. 1. Examples of the catatonic-like state produced by LSD in reserpinized cats. *Upper left.* Cats placed as shown (A) remained in place as the body descended very slowly in a horizontal plane to the mouth of the wastebasket (B). *Upper right.* Cats placed with the forefeet slightly ahead of the hindfeet and back arched (A) maintained this position as the head descended during the course of about 20 minutes until the nose or chin rested against the floor (B). *Lower left.* Cats placed in this position maintained this posture indefinitely. *Lower right.* Cats placed as shown here remained in position for varying lengths of time because of the difficulty in balancing them. In no case did an animal change position voluntarily. Those animals which tumbled into the basket remained in the position they assumed upon coming to rest.

amine could be said to produce a "chemical sympathectomy" along with other unknown effects, these experiments were necessary to evaluate differences in the effects of hexamethonium and phenoxybenzamine. Both of these compounds prevented the signs of sympathetic discharge seen in LSD treated animals, but only phenoxybenzamine was effective in altering the behavior pattern of animals so treated.

LSD in these animals produced a behavior pattern indistinguishable from that seen in control animals as shown in figure 2. It was apparent in these experiments that LSD may cause some delayed release of catecholamines from the denervated adrenal medulla. Upon injection of the LSD in these cats some piloerection was evident toward the tip of the tail, but all the body fur remained smooth until quite late in the reaction when minimal to moderate piloerection of the body became evident.

Phenoxybenzamine, 5.0 mg/kg i.v. 60 minutes prior to LSD, in this group of cats influenced the LSD reaction in a manner comparable to that seen in intact cats receiving 15 mg/kg. It would appear from these experiments that phenoxybenzamine antagonized the LSD effect through some pharmacologic effect other than peripheral sympathetic blockade, more than likely a central effect.

As with phenoxybenzamine, hydralazine, 10 mg/kg i.v. 10 minutes before LSD, now produced a significant attenuation of the LSD reaction. Figure 2 illustrates the effects of these experiments.

DISCUSSION. The failure of atropine and

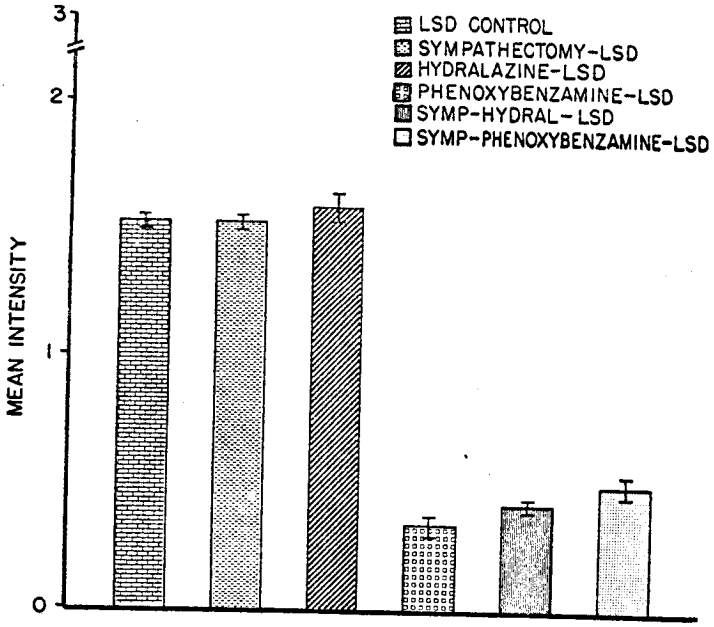


FIG. 2. Effects of sympathectomy, hydralazine and phenoxybenzamine (Dibenzyline) on the LSD reaction.

The reaction to LSD in sympathectomized animals was no different from that in control animals. Hydralazine had no effect upon the LSD reaction in intact animals, but was quite effective in sympathectomized animals. Phenoxybenzamine (Dibenzyline), 15 mg/kg, produced a marked change in the reaction in intact cats, as did 5.0 mg/kg in the sympathectomized animals. At least 4 animals were used in each experimental procedure. The LSD control represents 30 animals. The vertical lines indicate the standard error of the mean for each group.

scopolamine to prevent the behavioral effects of LSD would indicate that cholinesterase inhibition is not an important facet of the mechanism of action of LSD (Poloni and Maffezzoni, 1952; Greig and Gibbons, 1959).

The lack of effect of hexamethonium and total sympathectomy indicates that there is no causal relationship between the peripheral autonomic system and the LSD-induced behavior pattern.

Chlorpromazine has been reported by many investigators to inhibit the effects of psychotomimetic agents in humans and animals (Elkes, 1956; Shore and Brodie, 1957; Isbell and Logan, 1957; Bradley and Hance, 1957). It appears that the hypothalamic depression it produces is important to the tranquilizing effect of the drug (Hiebel *et al.*, 1954a, b; Dasgupta and Werner, 1954; Jourdan *et al.*, 1955; Moran and Butler, 1956; Spector *et al.*, 1957) and this could be the primary site of action of this antagonist.

Since these experiments were first carried out, several investigators have reported that reserpine potentiates or at least fails to antagonize the effects of LSD (Gaddum and Vogt, 1956; Isbell and Logan, 1957; Gogerty *et al.*, 1957). In any interpretation of the effects of reserpine, serotonin (5-HT) and catecholamines must be considered. Most likely 5-HT is the more important, since the behavioral responses to reserpine may be altered by prior treatment with a monoamine oxidase (MAO) inhibitor. Indeed, agitation may be produced by giving 5-hydroxytryptophan (5-HTP) in the presence of an MAO inhibitor (Brodie and Shore, 1957).

Since surgical sympathectomy and ganglionic blockade did not affect the LSD-induced behavior, we can attribute the efficacy of phenoxybenzamine only to depression of areas of the CNS concerned in this reaction. The fact that phenoxybenzamine achieves appreciable concentrations in the CNS (Brodie *et al.*, 1954) and has been shown to exert demonstrable effects upon centrally influenced reflexes (Cranmer and Bach, 1957; Sigg *et al.*, 1955) tends to make this a tenable conclusion.

SUMMARY

Of the drugs scopolamine, atropine, hexamethonium, reserpine, hydralazine, phenoxybenzamine and chlorpromazine, only phenoxybenzamine and chlorpromazine were effective as antagonists of the behavioral effects induced by LSD.

Surgical sympathectomy and adrenal demedullation did not affect the behavior pattern induced by LSD.

Reserpine did not prevent the behavioral changes induced by LSD, but caused the animals so treated to develop a catatonic-like state immediately the typical behavior pattern ceased.

It is concluded that chlorpromazine and phenoxybenzamine are effective through depression of central sympathetic control areas.

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