Proc.Soc.exp.Biol. (N.Y.) 102, 697-701 (1959).

LSD 806

ect of Lysergic Acid Diethylamide and Bufotenine on Performance of Trained Rats. (25367)

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psychogenic activity of lysergic acid ylamide (LSD) has long been recognized. been reported that a condition resembling schizophrenia can be produced in humans by administration of only 1 μg of LSD/kg(1). Winter and Flataker(2) demonstrated that

the effects of LSD upon trained rats bear a linear relationship to the log dose of the drug. These authors also reported a prolongation of climbing time of trained rats following injections of plasma from psychotic patients(3). Various other substances such as harmine, yohimbine and mescaline(4,5), have been used to produce abnormal mental conditions. Fabing and Hawkins(6) reported bufotenine to cause visual hallucinations in man and a bizarre syndrome of activity in rats and dogs. Since the endogenous production of bufotenine in humans remains a possibility(7), it was considered important to examine the psychogenic activity of bufotenine more closely. In the present report a comparison was made between psychogenic effects produced in trained rats by administration of LSD and bufotenine.

Methods and materials. The technic used for evaluating alterations in mental activity after administration of psychogenic drugs was similar to that described by Winter and Flataker(2). It consisted of measuring the delay in climbing time of trained rats: which in the present study were trained to climb a 5 foot, vertical, cloth covered pole rather than a rope. Rats of either sex weighing 100-250 g were used. Since fasting the animals did not appear to influence the outcome of these studies, they received food and water *ad lib.* All drugs were injected intraperitoneally (I.P.). Solutions were made fresh as needed. In those experiments where attempts were made to alter the effect of LSD or bufotenine, the animals were pretreated with reserpine,* 5-hydroxytryptophan (5-HTP) and iproniazid* (1-isonicotinyl-2-isopropylhydrazine) at 30, 90 and 180 minutes respectively. Injections of iproniazid are expressed as its phosphate salt, bufotenine as its mono-oxalate H_2O complex and 5-hydroxytryptophan as the free amino acid; reserpine as its phosphate salt.

Results. Effect of LSD and bufotenine on trained rats: Both LSD and bufotenine caused prolongation of climbing time of trained rats. The quantitative aspects of this effect are shown in Fig. 1. Here total seconds

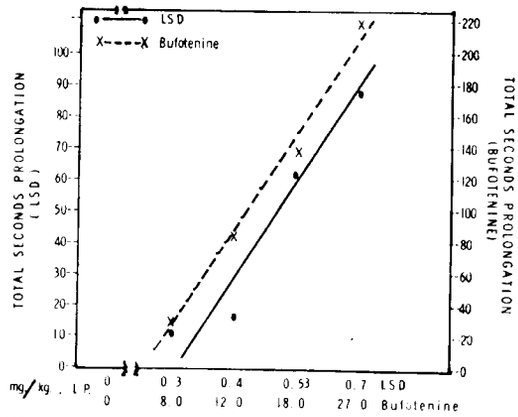

FIG. 1. Total seconds prolongation of trained rats relative to log dose of LSD and bufotenine.

prolongation is plotted against amount of drug injected. In Table I results are recorded in terms of C.T.D. (climbing time delay). Expressed either way the results show a direct proportionality between amount of drug administered and its effect on behavior of these rats.

There are differenecs as well as similarities in the effects of LSD and bufotenine intoxication. With either drug the action becomes apparent within 5-10 minutes after administration. However, with LSD the effect of the drug wears off in about 20 minutes. With bufotenine, on the other hand, intoxication

TABLE I. Climbing Time Delay for 5 Trained Rats per Dose after Psychogenic Drug Administration.

Drug	mg/kg IP	C.T.D.†	Observation, min.
1. LSD	.0*	.0	30
	.3	58.5	"
	.4	101.0	"
	.53	383.5	"
	.70	426.5	"
2. Bufotenine	.0*	124.0	120
	8.0	330.0	"
	12.0	746.5	"
	18.0	1667.5	"
	27.0	2397.5	"
3. (Bufotenine tolerant rats)			
LSD	.53	.0	30
4. (LSD tolerant rats)			
Bufotenine	27.0	26.5	60

* Saline inj.

† C.T.D. value takes into consideration the intensity and duration of prolongation in climbing time; computed according to Winter and Flataker(2).

* Reserpine used was donated by Ciba Pharmaceutical Products, Summit, N. J., Iproniazid donated by Hoffmann-La Roche, Nutley, N. J.

may last as long as 90-120 minutes. Thus while both LSD and bufotenine affect behavioral characteristics of the test animal, LSD produces its effect at a much lower dose than does bufotenine. The confused state brought about by either drug often caused animals to climb only part way up and return to the stimulating grid instead of making a systematic ascent to the platform. This striking phenomenon is difficult to quantitate. Administration of either LSD or bufotenine produced a peripheral vasodilation as evidenced by bright red color of ears and paws. The effects of the 2 drugs differed in that LSD produced hypersalivation and increased frequency of defecation and urination, whereas these effects were not observed following bufotenine injection. This phase of the study substantiates results of Winter and Flataker(2) with respect to LSD. However, the effect of administered psychotic plasma to such animals, as observed by these authors(3), could not be confirmed.

Tolerance to bufotenine and LSD: A tolerance to the behavioral and pyretogenic responses to LSD has been reported(8). During our study, after 4 or 5 injections of bufotenine the rats not only became refractory to further injections of this drug, but developed a tolerance to LSD as well (Table I-3). Similarly, rats made refractory by several injections of LSD became tolerant to both LSD and bufotenine (Table I-4).

Potentiation of LSD and bufotenine effect by reserpine: Winter and Flataker(2) reported a potentiation of LSD effect by administration of reserpine. These findings could be confirmed in our study, although only a small number of animals were used. When reserpine (1.25 mg/kg) was administered along with LSD (0.3 mg/kg) the effect was greater than that of LSD or reserpine alone (Table II-1). A similar effect on the bufotenine action could also be demonstrated (Table II-2). Thus when reserpine (5 mg/kg) was given along with a minimally effective dose of bufotenine (8 mg/kg) the C.T.D. was increased several fold as compared to that obtained when a similar amount of bufotenine was injected alone. Since, as already mentioned, the rats developed a tolerance to repeated injection of the 2 psychogens, a new group of rats had to be used for each study. Any variations occurring as a result of this procedure were controlled by following a cross-over pattern of injection.

TABLE II. Alteration of Climbing Time Delay of LSD and Bufotenine Treated Rats.

	Drug, mg/kg, IP		No. of animals per dose	C.T.D.	Avg duration of drug action, min.
1.	Reserpine	LSD			
		.3	1		60
	1.25	.3	1	326	"
	1.25		2	62	"
2.		Bufotenine			
		8	2	818	120
	5	8	2	6780	"
	5		2	994.5	"
3.	5-HTP	LSD			
		.5	5	943.9	40
	100	.5	5	443.5	"
4.		Bufotenine			
		27	4	2467	60
	100	27	4	1483	"
5.		LSD			
		.3	5	77.5	40
	100	.3	5	173.3	"
6.		Bufotenine			
		12	5	450.5	60
	100	12	5	842.7	"
7.	Iproniazid	LSD			
		.3	3		30
	120	.3	4	172.7	"
8.		.5	5	252.7	"
	120	.5	6	1344	"
9.		Bufotenine			
		12	5	26.3	60
	120	12	6	1186	"

Alterations in psychogenic activity of drugs as a function of brain serotonin levels. Experiments were carried out to illuminate the possible effect of increased brain levels of serotonin on the action of the psychogenic drugs. Animals were pretreated with 5-hydroxytryptophan (5-HTP) or with iproniazid before receiving the psychogenic drugs. Ample evidence has been reported that administration of either 5-HTP or iproniazid(9) will raise the brain level of serotonin (5-HT), hence no actual determinations of brain 5-HT were carried out. Injection of trained rats with 100 mg/kg 5-HTP produced no detectable change in behavior of these animals. However, when

such administration of 5-HTP was followed 90 minutes later by an injection of 0.5 mg/kg LSD, the usual response to such a dose of LSD was reduced considerably (Table II-3). But when a group of similarly 5-HTP pretreated rats were injected with only 0.3 mg/kg LSD a prolongation rather than a reduction of C.T.D. was observed (Table II-5). These experiments were repeated substituting bufotenine for LSD. When rats pretreated with 100 mg/kg 5-HTP were injected with a relatively large dose of bufotenine a reduction in C.T.D. was produced (Table II-4). If the 5-HTP pretreated rats were injected with a smaller dose of bufotenine (Table II-6) the C.T.D. was increased.

Another method for increasing the level of serotonin in the brain relies on inhibition of monoamine oxidase system by iproniazid. One would expect, therefore, that pretreatment of rats with iproniazid prior to injection of LSD or bufotenine should produce effects similar to those seen after pretreatment with 5-HTP. As Table II shows this is actually the case. Pretreatment with iproniazid increased the C.T.D. in every case with either LSD or bufotenine. Here again similarity in action for these 2 psychotropic drugs is demonstrated.

Discussion. The method of Winter and Flataker(2) was used in our study, because it has features making it particularly suitable for this type of work. Thus it is neither difficult nor tedious to train rats to climb a pole in a reproducible length of time. This was of particular value, since the animals quickly developed a tolerance and cross-tolerance for the 2 drugs (LSD and bufotenine), hence they could be used only for a limited number of trials. The disadvantages of the pole climbing technic for assaying psychogenic drugs are also quite apparent. Ability of a rat to climb a 5 foot pole in a reasonable length of time depends upon 2 factors: (a) a mental factor, the will to climb, and (b) a muscular factor, the strength necessary to do so. Hence any results obtained by this method should be interpreted with some caution.

That these psychogenic drugs produce their effect by interfering with normal serotonin metabolism seems particularly attractive(10). In the present study the effects of 2 serotonin analogs, LSD and bufotenine were compared. Both of them are capable of producing confusion and behavioral changes in trained rats. Upon repeated injections of each of these 2 drugs the rats readily developed a tolerance to them. Even more interesting is the observation that rats made refractory to one of these drugs also became tolerant to the other. This induced cross-tolerance strongly suggests that both LSD and bufotenine produce their psychogenic effect by the same mechanism. Any treatment known to affect serotonin metabolism and which can be shown to modify the action of both LSD and bufotenine would lend support to this concept.

Reserpine has definite tranquilizing properties and also causes a release of bound serotonin in the brain and other tissues(11). Hence increased C.T.D. shown in Table II when reserpine was administered along with either LSD or bufotenine could well be related to its interference with bound serotonin level in the brain.

More clear cut results were obtained when procedures known to increase the level of brain serotonin were employed. Iproniazid has been shown to block the monoamine oxidase system, which probably is the main pathway for normal catabolism of serotonin. Prior administration of iproniazid greatly potentiates the action of both LSD and bufotenine (Table II). Similarly pretreatment of rats with 5-hydroxytryptophan potentiates the effects of LSD and bufotenine when administered in doses of 0.3 and 12 mg/kg respectively. However, an anomaly occurred with both LSD and bufotenine. With prior administration of 100 mg/kg 5-HTP the effect of small doses of both psychogenic drugs was enhanced. The effect of larger doses of drugs on the other hand was reduced by prior administration of 5-HTP. Further studies are required to illuminate this finding. Yet the interesting feature is that the result of 5-HTP pretreatment modifies the effect of LSD and bufotenine in a similar manner, thus strengthening the hypothesis that these 2 drugs produce their psychogenic action through the same mechanism or target cells.

Summary. 1. A comparative study has

been carried out to determine the effect of LSD and bufotenine on climbing time of trained rats. 2. Both drugs produced similar effects in these animals. However, LSD was effective at smaller doses but had a shorter duration of action than bufotenine. 3. Tolerance to repeated LSD injections was found and a similar tolerance to bufotenine was demonstrated. 4. Prolongation of climbing produced by submaximal doses of both LSD and bufotenine was potentiated when brain serotonin levels were altered by pretreatment of animals with reserpine, 5-HTP or iproniazid. Highly effective doses of LSD and bufotenine were inhibited by 5-HTP pretreatment. 5. Based on evaluation of climbing time delay produced in trained rats the psychogenic activity of LSD and bufotenine appears to depend on a similar mechanism of action.

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Received September 14, 1959. P.S.E.B.M., 1959, v102.

Effect of Glucagon on Renal Functions in the Dog.* (25368)

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Glucagon, in addition to effects on carbohydrate metabolism(1) has been reported to increase urinary excretion of electrolytes in dog (2) and in man(3,4). These authors agree that the electrolyte effects of glucagon are independent of its hyperglycemic action. They also feel that the effects of glucagon on electrolyte excretion probably result from a direct action on the renal tubule since they observed only occasional or slight increases of equivocal significance in glomerular filtration rate (GFR) and renal plasma flow (RPF). Glucagon does not alter peripheral blood flow as measured by finger plethysmography(5), but does produce a marked increase in hepatic blood flow(6). In view of the equivocal data available regarding glomerular filtration rate and renal plasma flow after glucagon administration, and since small changes in glomerular filtration rate could produce large changes in electrolyte excretion, this problem has been further investigated in the dog. In addition, the effect of glucagon on maximum rate at which the renal tubules can reabsorb glucose (Tm glucose) was investigated. In a recent study(7), glucagon was found to have no effect on Tm_G in 2 volunteer subjects.

Materials and methods. Seven healthy trained unanesthetized female mongrel dogs between 45 and 55 lb in weight were used in this study. Exogenous creatinine clearance (GFR), p-amino hippurate (PAH) clearance (RPF) and Tmglucose (Tm_G) were measured by standard technics(8,9). Briefly, creatinine and PAH or creatinine and glucose were administered at a constant rate by means of an infusion pump. After a suitable period allowed for equilibrium, urine was collected by catheter. Each collection period was terminated by a bladder rinse with distilled water. Urine collection periods were accurately timed. Blood was obtained at mid-

* Aided by grants from U. S. Public Health Service and Otho S. A. Sprague.