Neuropharmacology, 1973, 12, 471–476

THE EFFECTS OF DL-α-METHYLTYROSINE AND L-DOPA ON THE HYPERTHERMIC AND BEHAVIORAL ACTIONS OF LSD IN RABBITS*

A. HORITA and A. E. HAMILTON

Department of Pharmacology, University of Washington, Seattle, Washington 98195

(*Accepted* 12 *October* 1972)

Summary—Lysergic acid diethylamide (LSD) in relatively small doses produces in rabbits a dose-related hyperthermia and behavioral excitation.

After a 24 hr pretreatment with DL-α-methyl-*p*-tyrosine (α-MT), the LSD hyperthermia is no longer dose related. With lower doses of LSD it is potentiated, and with higher doses attenuated. The behavioral actions of LSD are attenuated at all dose levels.

The administration of L-dihydroxyphenylalanine (L-DOPA) restores the behavioral action of LSD in α-MT-pretreated rabbits. Prior administration of the dopamine-β-hydroxylase inhibitor, sodium diethyldithiocarbamate (DEDC) was ineffective in blocking the restorative action of L-DOPA.

Analyses of brainstem catecholamines indicate that under the conditions of this study α-MT markedly depletes brain norepinephrine (NE) and dopamine (DA). L-DOPA restores DA and partially restores NE levels to control values. In animals pretreated with DEDC the effect of L-DOPA treatment on brainstem DA is enhanced with only slight increases in NE levels.

Lysergic acid diethylamide (LSD-25), the well known hallucinogen, was earlier demonstrated as a potent hyperthermic agent, especially in rabbits (HORITA and DILLE, 1954). Accompanying this temperature response are the signs of behavioral excitation and peripheral sympathetic stimulation. HORITA and GOGERTY (1958) compared the hyperthermic and excitatory effects of LSD with those of 5-hydroxytryptamine (5-HT) as induced by administration of 5-hydroxytryptophan (5-HTP) and concluded that LSD mimicked the actions of increased levels of 5-HT in the central nervous system. Nevertheless, the possibility of adrenergic mechanisms in the actions of LSD could not be precluded. In fact, some of the earlier experimental evidence suggested that catecholamines might be involved in the hallucinogenic action of LSD in man (ROTHLIN, CERLETTI, KONZETT, SCHALCH and TAESCHLER, 1956; HOAGLAND, 1957).

Recently, with the aid of the tyrosine hydroxylase inhibitor, DL-α-methyl-*p*-tyrosine (α-MT), we were able to demonstrate that the hyperthermic and the behavioral effects of LSD could be dissociated. Only the behavioral effect was attenuated or abolished after α-MT, but the hyperthermic response was either unchanged or enhanced after brain norepinephrine (NE) levels were lowered (HORITA and HAMILTON, 1969).

The present study is an extension of these observations, and data regarding different dose levels of LSD, as well as the possible role of brain dopamine in the LSD response, are presented.

* Supported in part by U.S. Public Health Service grant MH-02435.

METHODS

Male albino rabbits, 1·5–2·5 kg, were placed in open stanchions as described by SHELLENBERGER and ELDER (1967). Animals were so restrained for 6–8 hr per day for 2 days prior to drug injection in order to stabilize both behavior and basal temperature. On the third day pretreatment of experimental animals was begun by administering i.p. 80 mg/kg of α-MT suspended in water. These injections were made every 4 hr for 24 hr. Temperatures were monitored over an 8 hr period of treatment with α-MT. At the end of this time the animals were returned to their cages and provided food and water. On the following morning the rabbits were placed in the stanchions and both controls and pretreated animals received varying doses of LSD i.v. Colonic temperatures were measured by a YSI Model 47 telethermometer and recorded on a Leeds-Northrup Speedomax multichannel recorder. Gross behavior and sympathetic activity were determined mainly by observations of motor activity, rate of respiration, pupillary dilatation, and skeletal muscle tone.

The effect of L-dihydroxyphenylalanine (L-DOPA) on the antagonism of LSD by α-MT was also investigated. In such experiments rabbits were first pretreated with α-MT for 24 hr as described above. LSD was then given, and 30 min later L-DOPA in a dose of 10 mg/kg was administered i.v. In other animals L-DOPA was given 30 min before the LSD.

In order to determine whether dopamine (DA) or NE was involved in the LSD effect on behavior we treated several animals with sodium diethyldithiocarbamate (DEDC), 200 mg/kg, 1–2 hr after the final dose of α-MT. To these animals L-DOPA and LSD were given, and temperatures, behavior and catecholamine levels were determined.

Brainstem DA and NE were determined by the method described by SHELLENBERGER and GORDON (1971). The animals were generally sacrificed 1–2 hr after the last injection of α-MT by injecting 30 ml of air into the marginal ear vein. In those studies involving L-DOPA and DEDC both DA and NE assays were made on brainstems of animals sacrificed 1–2 hr after the L-DOPA administration. The catecholamine levels are expressed as μg/g of wet tissue.

RESULTS

Effect of LSD in normal and α-MT-pretreated rabbits

The administration of 100–300 μg/kg of LSD to normal rabbits resulted in a hyperthermia which peaked within the first 1–2 hr and gradually returned to normal over the following 5–7 hr (Fig. 1A). These responses were generally dose-dependent, ranging from about 1·5°–3·0°C above control levels. In most instances, those animals reaching 42°C or above died at the peaks of the temperature response. Accompanying the hyperthermia were a number of grossly observable signs, such as increased motor activity, excitation, pupillary dilatation, piloerection and increased skeletal muscle tone.

A dose of 100 μg/kg produced a moderate increase in respiratory rate, motor activity, and general excitability along with marked pupillary dilatation and exophthalmia. As the dose was increased to 200 and 300 μg/kg the previously mentioned signs became more pronounced and additional signs of salivation, piloerection, hyperextension of the hind legs and loss of motor control occurred. Also at the higher doses a "raspy" type of respiration was often present, apparently due to the fluid accumulations in the respiratory tract. As the response of the rabbits to 200 μg/kg, and especially 300 μg/kg, progressed, the motor activity degenerated into generalized muscle tremors and ataxia with convulsions often present prior to death.

Rabbits which received 7 i.p. injections of 80 mg/kg dose of α-MT exhibited a somewhat sedated appearance, although by no means did they demonstrate ataxia or motor incoordination. When permitted to walk about freely they were hardly distinguishable from control animals. Upon placing them under restraint (as described in Methods) they eventually exhibited eyelid closure and a light sleep-like state, from which they were easily aroused. Basal rectal temperatures of such animals were essentially the same as normal animals, ranging between 38·5° and 39·5°C. The administration of 100, 200 and 300 μg/kg of LSD to α-MT-pretreated rabbits resulted in the prompt hyperthermic response, but in this instance there appeared to be a loss of a dose–response relationship in the intensity of the temperature response (Fig. 1A).

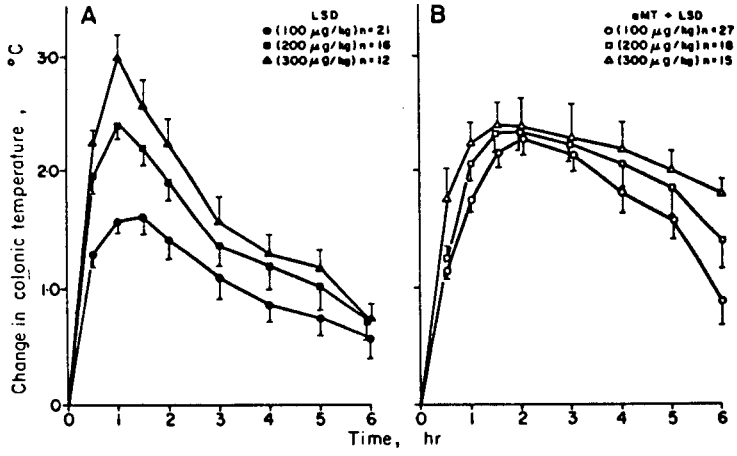

Fig. 1. Changes in rectal temperature upon treatment with LSD in (A) controls and (B) α-MT pretreated rabbits. Vertical lines indicate the standard errors of mean temperature values.

A comparison of the hyperthermic response in control LSD animals and α-MT-LSD animals points to several differences (Fig. 1B). In the latter animals, given a 100 μg/kg dose of LSD, the hyperthermic response was potentiated both in intensity and duration, and peak effects were reached about an hour after that seen in control rabbits receiving the same dose of LSD. With 200 μg/kg doses of LSD both control and α-MT-pretreated rabbits reached about the same degree of hyperthermia, although the peak response, and especially the rate of recovery, were again delayed. In control animals a dose of 300 μg/kg of LSD was extremely toxic, and temperatures usually exceeded 42°C. The animals were extremely agitated, excited, and presented signs of excessive sympathetic stimulation. Of the 12 animals employed in this study at this dose of LSD, 5 died at the peak of the hyperthermia. In α-MT-pretreated animals 300 μg/kg of LSD produced an attenuated temperature response, although again, the duration of response was much prolonged over that seen in the control-LSD animals. The death rate was also less in these pretreated animals in that only 3 of 15 died. An analysis of variance of the 6 temperature curves in Figure 1 reveals that in control rabbits the temperature responses differed significantly ($P < 0{\cdot}05$) among the three dose levels up to 2·5 hr after administration, while in the α-MT-pretreated rabbits significant differences were seen only during the first hr. Comparison of the LSD curves of control vs

α-MT-pretreated animals also indicates significant differences in the extent of hyperthermia over time, especially with the 100 and 200 μg/kg doses of LSD. At these dose levels significant differences were present up to 5·5 hr. At the 300 μg/kg dose of LSD a significant difference in temperature response between control and α-MT-pretreated animals was obtained for only the first hour. The variation in hyperthermia at this high dose was considerable, for it was approaching the LD_{50} of LSD.

The effect of α-MT-pretreatment on the behavioral action of LSD was quite dramatic. In such animals 100 μg/kg of LSD produced only some increase in respiration and muscle tone, but pupillary dilatation, excitation and piloerection were essentially absent. When the doses of LSD were raised to 200 and 300 μg/kg more breakthrough of the excitatory signs were observed. Gross measurements of behavior indicated that in α-MT-pretreated rabbits 300 μg/kg of LSD produced about the same intensity of signs as that seen with 100 μg/kg in control rabbits. Thus, a marked attenuation of the behavioral action of LSD was produced by α-MT-pretreatment.

In order to establish the effectiveness of α-MT-pretreatment in lowering brain NE levels, NE assays were carried out in brainstems of control and α-MT-pretreated rabbits. Consistent with the findings of other investigators, brainstem NE levels of α-MT-pretreated rabbits were reduced, decreasing to 0·07 ± 0·03 μg/g of brain as compared to the mean of 0·58 ± 0·06 μg/g found in control animals.

Effect of L-*DOPA on the behavioral response to LSD in control and α-MT-pretreated rabbits*

Because α-MT attenuated markedly the behavioral actions of LSD we decided to determine whether L-DOPA (10 mg/kg i.v.) would restore this effect by regenerating brain catecholamines. L-DOPA in the dose employed did not alter normal behavior nor that of the α-MT-pretreated rabbit. Also, L-DOPA did not affect the LSD response in control rabbits. However, if DOPA was given 15–30 min prior to or after the administration of LSD, the behavioral action to LSD was again restored. Total restoration was not evident, but the most prominent effects were the pupillary dilatation, exophthalmia, increased alertness and some excitation.

Failure of DEDC to block the L-*DOPA reactivation of LSD response in α-MT-pretreated rabbits*

DEDC, administered in doses of 200–300 mg/kg 1–2 hr prior to L-DOPA, did not alter the onset or intensity of the behavioral effects of LSD in α-MT-pretreated rabbits. DEDC alone was without effect on behavior of normal or α-MT-pretreated animals.

In order to establish the degree of inhibition of NE formation by DEDC and the extent of DA increase that occurred with L-DOPA administration, brainstems were analyzed for DA and NE. Results in Figure 2 indicate that 2 hr after L-DOPA brainstem DA levels of α-MT-pretreated rabbits were restored to normal. NE levels are also raised but to a much lesser extent at this time. Administration of DEDC 1 hr prior to L-DOPA resulted in a similar increase in DA levels, but the increase in NE was attenuated some 50% of those not treated with DEDC. Total inhibition of NE synthesis with DEDC was not possible, even when the dose of DEDC was raised to 400 mg/kg. The extent of DA and NE increase after DEDC and L-DOPA was not affected by subsequent administration of LSD (Fig. 2).

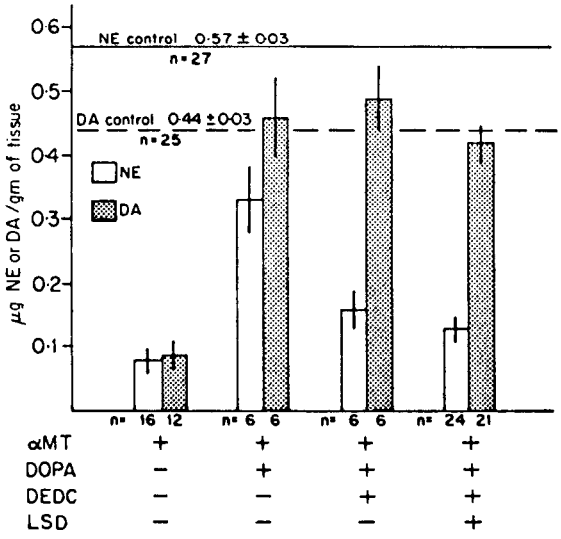

Fig. 2. Norepinephrine (NE) and dopamine (DA) levels of brainstems in control and drug treated rabbits. Vertical lines indicate standard errors of mean values.

DISCUSSION

Our earlier report on the interaction between α-MT and LSD in the rabbit indicated that depletion of catecholamines via tyrosine hydroxylase inhibition did not affect the hyperthermic action of LSD. However, our previous studies were made with the α-MT suspended in a 10% solution of polyethylene glycol. Although solvent controls were run we subsequently discovered a partial antagonistic action of the suspending medium on the LSD response in α-MT-pretreated animals. In the present study the α-MT was suspended in distilled water, and it is apparent that some modification of the LSD hyperthermia is produced by pretreatment with α-MT.

The conversion of the dose-related hyperthermia of LSD to that of a nongraded response is paradoxical. Within the range of 100–300 μg/kg of LSD in the α-MT-pretreated rabbit the hyperthermia does not differ significantly at any time during the course of the response, while in control rabbits the differences in temperature rise among the three dose levels were highly significant during the first 3 hr. The reasons for this change in hyperthermic response to LSD after α-MT is not apparent from these experiments. It is indicative of an adrenergic contribution to the normal response to LSD, but adrenergic mediation is not necessary for the genesis of the hyperthermia. This suggests that a mechanism other than that involving catecholamines may be responsible for the hyperthermic action of LSD, and supports our earlier hypothesis that it may be one of mimicking the action of an excess concentration 5-HT in the CNS. It is also consistent with the current views of FELDBERG and MYERS (1964) who postulate a possible role of 5-HT in temperature regulation.

Behaviorally, it is obvious that a marked antagonism to LSD was exerted by pretreatment with α-MT. Thus, as described previously, α-MT causes a dissociation of the behavioral and hyperthermic effects of LSD. The attenuation of the behavioral response to LSD is especially marked when lower doses of the hallucinogen (100 μg/kg) are used. At the higher doses (300 μg/kg) the behavioral and sympathomimetic actions of LSD again become

manifest. This "breakthrough" response may indicate the incompleteness of the α-MT-induced depletion of catecholamines, or it may mean that at higher doses LSD produces a direct action not related to endogenous stores of catecholamines.

The fact that L-DOPA restores to a large extent the behavioral actions of lower doses of LSD in α-MT-pretreated rabbits indicates that catecholamines are possibly involved in the genesis of this response. The DEDC-DOPA experiments further suggest that DA, rather than NE, might be the primary intermediate in the behavioral action of LSD in the rabbit.

Other authors (SUGRUE, 1969; APPEL, LOVELL and FREEDMAN, 1970) have found α-MT to be ineffective in modifying the LSD-induced behavioral effects. However, their experiments were carried out in rats, and the α-MT dose and time schedules were greatly different from ours. We find that in order to antagonize the behavioral action of LSD in rabbits and to lower brain NE and DA to very low levels, it is necessary to employ the repeated administration of α-MT over a 24-hr period. A single dose of 100 mg/kg of α-MT 5 hr prior to LSD does not modify significantly the behavior pattern to the hallucinogen.

The present study has demonstrated the ability of α-MT to dissociate the hyperthermic from the behavioral actions of LSD in the rabbit. It supports the view that at least at the lower doses the behavioral actions of LSD are mediated via catecholamines, but that the hyperthermic response, while modified by α-MT, is not directly related to the brainstem levels of catecholamines.

REFERENCES

APPEL, J. B., LOVELL, R. A. and FREEDMAN, D. X. (1970). Alterations in the behavioral effects of LSD by pretreatment with *p*-chlorophenylalanine and α-methyl-*p*-tyrosine. *Psychopharmacologia* **18**: 387–406.

FELDBERG, W. and MYERS, R. D. (1964). Effects on temperature of amines injected into the cerebral ventricles. A new concept of temperature regulation. *J. Physiol., Lond.* **173**: 226–237.

HOAGLAND, H. (1957). A review of biochemical changes induced *in vivo* by lysergic acid diethylamide and similar drugs. *Ann. N.Y. Acad. Sci.* **66**: 445–458.

HORITA, A. and DILLE, J. M. (1954). Pyretogenic effect of lysergic acid diethylamide. *Science* **120**: 1100–1101.

HORITA, A. and GOGERTY, J. H. (1958). The pyretogenic effect of 5-hydroxytryptophan and its comparison with that of LSD. *J. Pharmac. exp. Ther.* **122**: 195–200.

HORITA, A. and HAMILTON, A. E. (1969). Lysergic acid diethylamine: dissociation of its behavioral and hyperthermic actions by DL-α-methyl-*p*-tyrosine. *Science* **164**: 78–79.

ROTHLIN, E., CERLETTI, A., KONZETT, H., SCHALCH, W. R. and TAESCHLER, M. (1965). Zentral vegetative LSD-effekte. *Experientia* **12**: 154.

SHELLENBERGER, M. K. and ELDER, J. T. (1967). Changes in rabbit core temperature accompanying alterations in brainstem monoamine concentrations. *J. Pharmac. exp. Ther.* **158**: 219–226.

SHELLENBERGER, M. K. and GORDON, J. H. (1971). A rapid, simplified procedure for simultaneous assay of norepinephrine, dopamine and 5-hydroxytryptamine from discrete brain areas. *Analyt. Biochem.* **39**: 356–372.

SUGRUE, M. F. (1969). A study of the role of noradrenaline in behavioral changes produced in the rat by psychotomimetic drugs. *Br. J. Pharmac.* **35**: 243–252.