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6. We thank Dr. D. H. Moore, Institute for Medical Research, Camden, New Jersey, for supplying ten of these milk samples.

30 January 1969

Lysergic Acid Diethylamide: Dissociation of Its Behavioral and Hyperthermic Actions by DL-α-Methyl-p-Tyrosine

Abstract. *Rabbits treated with LSD 25 exhibit characteristic signs of hyperexcitability, increased peripheral sympathetic activity, and hyperthermia. When the rabbits received prior treatment with* DL-α-*methyl*-p-*tyrosine, the excitation and sympathetic actions of LSD 25 were abolished or attenuated, but the hyperthermia was unchanged from that of the controls. Concentrations of norepinephrine in brain stems of treated rabbits were greatly decreased. The excitation of central nervous system and sympathomimetic actions of LSD 25 in the rabbit are apparently mediated by norepinephrine, whereas the hyperthermic action functions through a nonadrenergic mechanism.*

In experimental animals lysergic acid diethylamide (LSD 25) produces a dose-related hyperthermia (*1*). In rabbits this response is accompanied by physiological and behavioral changes, such as increased motor activity, excitation, pupillary dilatation, and hypersalivation. This excitatory syndrome (*2*), including the hyperthermia, is in some ways similar to the psychic effect in man (*3*). Although the mechanism of behavioral action of LSD 25 is unknown, it has been suggested by indirect evidence only that the catecholamines might take part in an intermediate step (see *4*).

The inhibitor of tyrosine hydroxylase, DL-α-methyl-*p*-tyrosine (*5*) (α-MT), when given in repeated doses, causes the depletion of norepinephrine in a variety of tissues by inhibiting the conversion of tyrosine to dihydroxyphenylalanine. If norepinephrine is involved in the excitatory syndrome, animals so treated should respond to LSD 25 in a modified manner.

Male albino rabbits (1.5 to 2.5 kg) were divided into six groups as follows: (i) those given only saline, (ii) those given polyethylene glycol solvent and saline, (iii) those given LSD 25, (iv) those given solvent and LSD 25, (v) those previously treated with α-MT and then given saline, and (vi) those previously treated with α-MT and then given LSD 25. Rabbits restrained in open stanchions (*6*) were intraperitoneally injected with α-MT (80 mg/kg), suspended in a 10 percent solution of polyethylene glycol 300, every 4 hours for 24 hours; LSD 25 was then given to control and previously treated animals, as well as to animals previously treated with solvent, and their behavior was recorded. Changes in rectal temperature were also monitored by a YSI model 47 telethermometer. Concentrations of norepinephrine in brain stems of animals treated with α-MT were compared with those of controls and of rabbits treated with solvent. Norepinephrine was determined by a modification of the method of Moore and Rech (*7*).

The intravenous injection of LSD 25 (100 μg/kg) to control rabbits results in pupillary dilatation, hyperventilation, increased motor activity, and an increase in rectal temperature (Fig. 1). Although the hyperexcitability subsides about 2 hours after administration of LSD 25, the hyperthermia gradually returns to normal in approximately 6 hours. Animals first treated with α-MT are sedated, but they exhibit functional reflexes and movements. The administration of LSD 25 to such animals results in very little behavioral change. A slight degree of increased respiration occurs in some cases, but generally the animals remain sedated. The effect of LSD 25 on rectal temperature, however, is present to the same extent as in the control animals; the peak response of 1.5° to 2.0°C occurs within 2 hours, the effect gradually subsiding within the subsequent 4 to 6 hours.

Between the rabbits that received only LSD and those treated with α-MT and LSD, there were no significant differences in temperature changes at any of the designated times, when analyzed by the *t*-test. Although the behavioral and sympathomimetic actions of LSD 25 were not affected in the controls that received polyethylene glycol, the hyperthermic response was slightly but consistently lower in those rabbits. Statistically, however, rabbits treated with the LSD and those treated with the glycol and LSD differed significantly ($P < .05$) only at the 15-minute and 4-hour readings. The nature of the apparent antipyretic action of polyethylene glycol is not known, but by itself it does not seem to produce a hypothermic action (Fig. 1). Rather, animals first treated with solvent tend to have slightly higher initial temperatures.

The amounts of norepinephrine in brain stems of controls and animals first treated with α-MT were assayed to

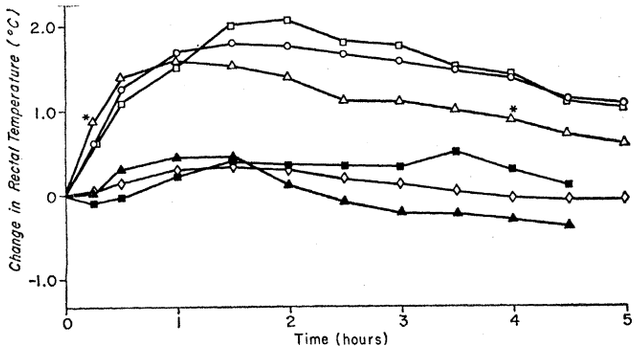

Fig. 1. Changes in rectal temperature in response to treatment with LSD in controls and rabbits first treated with α-MT. Solvent (10 percent polyethylene glycol 300) or α-MT suspended in solvent was injected intraperitoneally every 4 hours for 24 hours before administration of LSD or saline at zero time. Each curve represents the mean changes observed in 6 to 14 rabbits: (open circles) LSD ($n = 14$), (open triangles) solvent first then + LSD ($n = 10$), (open squares) α-MT first then LSD ($n = 9$), (open diamonds) saline ($n = 8$), (solid triangles) solvent first then saline ($n = 9$), and (solid squares) α-MT first then saline ($n = 6$). Asterisk denotes values which are significantly different from controls ($P < .05$).

determine the extent of norepinephrine depletion before LSD 25 administration. Brain stems of controls contained 0.58 ± .06 μg/g of tissue, whereas those of rabbits treated with α-MT contained 0.07 ± .03 μg/g. Treatment of animals with only the suspending medium for α-MT exerted no influence on concentrations of norepinephrine in the brain stem.

Our data indicate that both the excitation of the central nervous system and the peripheral sympathomimetic effects caused by LSD in the rabbit are related to the norepinephrine content of the brain. This conclusion is based on the fact that, after depletion of norepinephrine by α-MT, the marked behavioral actions of hyperexcitability, pupillary dilatation, increased motor activity, and so forth, are attenuated or abolished. The hyperthermic action of LSD 25, however, appears to be independent of brain catecholamines, for the temperature changes of controls and animals treated with α-MT in response to treatment with LSD are essentially identical. To our knowledge this is the first instance where it has been possible to separate so clearly the hyperthermic from the other central and peripheral actions of LSD 25. Other workers have employed various agents to antagonize this hyperthermia, but none has obtained the clear dissociation of the behavioral and temperature responses (*8*).

The possibility that LSD 25 exerts its hyperthermic action through a nonadrenergic mechanism is not incompatible with our earlier work in which we postulated a serotonin-like mechanism for the LSD 25 hyperthermia (*9*). The proposal by Feldberg and Myers (*10*) that serotonin may have a possible neurohumoral role in temperature regulation is also consistent with this view.

A. HORITA
A. E. HAMILTON
Department of Pharmacology,
School of Medicine,
University of Washington, Seattle 98105

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11. Supported by NIMH grant MH-02435.

13 January 1969

Ribonucleic Acid Biosynthesis in Adult and Infant Rat Brain in vitro

Abstract. *The rate of biosynthesis of ribonucleic acid (as judged by the rate of incorporation of uridine into ribonucleic acid) in infant and adult rat brain cortex slices, incubated aerobically in the presence of various substrates, is directly proportional to the adenosine triphosphate concentration. This suggests that the adenosine triphosphate concentration is one of the factors involved in the control of ribonucleic acid biosynthesis in infant and adult rat brain. Acetoacetate or β-hydroxybutyrate is about 70 percent as effective as glucose, with both infant and adult brain, for the promotion of ribonucleic acid biosynthesis, but they are considerably more effective than succinate in infant brain than in adult brain.*

In our experiments on the conditions for optimum rates of biosynthesis of RNA in the isolated brains of immature and adult animals, we have observed that the rate of biosynthesis of RNA in both adult and infant rat brain is directly proportional to the adenosine triphosphate (ATP) concentration in the presence of various substrates.

We prepared slices (0.3 to 0.4 mm thick) from the cerebral hemispheres of Wistar albino rats, using a Stadie-Riggs tissue slicer. The metabolism of the brain slices was investigated by the conventional Warburg manometric technique. The slices were weighed on a torsion balance and transferred to chilled manometric flasks; each flask contained 3 ml of Krebs-Ringer medium composed of 141 m*M* NaCl, 5.0 m*M* KCl, 2.3 m*M* $CaCl_2$, 1.3 m*M* $MgSO_4$, 10 m*M* Na_2HPO_4 (brought to *p*H 7.4 with 1.0*N* HCl), and 5 m*M* glucose. One dorsal and one lateral slice ("first slices"), weighing a total of 50 to 75 mg (wet weight), from the same brain were added to each flask. The glucose in the medium was omitted or replaced by other substrates, as occasion demanded. A solution of radioactive uridine (uridine-2-C^{14}) was placed in the side arm and tipped into the main vessel after its contents had been equilibrated at 37°C for 10 minutes in an atmosphere of oxygen. Incubation with the labeled uridine was carried out for periods of 30 minutes or 1 hour. Measurements of the rates of oxygen consumption were routinely made to ensure that the tissues exhibited their normal respiratory activities. Estimation of the labeled RNA was made by a modification of the technique of Schmidt and Thannhauser (*1*). The slices were removed after incubation, washed twice with ice-cold Krebs-Ringer medium to remove contaminating radioactivity, and homogenized in cold 5 percent trichloroacetic acid. The homogenate was kept at 0°C for 1 hour and centrifuged at 600*g* for 10 minutes; the residue was washed three times with 5 percent trichloroacetic acid to remove acid-soluble radioactive components. The lipids were removed by washing first with 95 percent ethanol and then with a mixture of 95 percent ethanol and ether (3 : 1); the residue was then suspended in 2 ml of 0.2*N* KOH and incubated at 37°C for 18 hours. Perchloric acid (0.05 ml of 70 percent) was then added to precipitate DNA and proteins. The supernatant contained the hydrolyzed RNA whose radioactivity was counted in the liquid scintillation counter. The concentration of ATP was estimated enzymatically by the use of hexokinase and glucose-6-phosphate dehydrogenase. The brain slices, after incubation, were homogenized in 2 ml of 6 percent perchloric acid and centrifuged, and 1.5 ml of the supernatant were neutralized with 5*M* potassium carbonate solution. After centrifugation at 0°C, 0.2 ml of the supernatant was assayed for ATP (*2*) using a flourimetric estimation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) concentration. Sodium acetoacetate solution was made fresh from ethyl acetoacetate by the method of Ljunggren (*3*).

Our results confirm the fact (*4*) that