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THE EFFECTS OF LSD UPON BRAIN INDOLEAMINE MATURATION IN THE BRAIN OF THE MOUSE

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Abstract—1. Mice either 1 or 7 days old were injected (i.p.) once with LSD in doses of 0·5 μg/kg, 5·0 μg/kg or 50·0 μg/kg, and were then killed for assay at 8 weeks of age.

2. Brains were assayed in four fractions (cerebellum, cerebral hemispheres, mesencephalon–diencephalon, and medulla-pons) for tryptophan-5-hydroxylase (T5-H), 5-hydroxytryptophan decarboxylase (5-HTPD), monoamine oxidase (MAO), 5-hydroxytryptamine (5-HT), and 5-hydroxyindole acetic acid (5-HIAA).

3. Seven day old mice receiving doses of 5·0 μg/kg and 50·0 μg/kg had a 10% elevation of 5-HT in the mesencephalon–diencephalon and a 10% elevation of MAO in the hemispheres.

4. The disruptive action at 7 day exposure correlates with the period of rapid terminal formation by the brain's 5-HT neurons.

INTRODUCTION

LYSERGIC acid diethylamide (LSD) has been implicated in developmental abnormality by two possible mechanisms. One involves chromosome breakage and raises the question of hereditary defect (Cohen *et al.*, 1967) while the other involves disruption of the developmental process with no hereditary overtones (Alexander *et al.*, 1967). A number of studies of both possible effects have appeared, and a variety of species have been used, including humans. Contradictory reports abound, and for studies of human subjects the number of other powerful drugs used are often numerous and cloud the issue (Long, 1972). Three comprehensive reviews of the literature have arrived at the same negative conclusions (Dishotsky *et al.*, 1971; Kalter, 1972; Long, 1972). Neither Dishotsky *et al.* (1971) nor Long (1972) find all the evidence convincing for either chromosomal breaks or developmental abnormality, and Kalter (1972) finds the evidence for abnormal development unconvincing.

One of the significant characteristics of LSD is its powerful antagonistic effect on the normal action of 5-hydroxytryptamine (5-HT). Because of its specific interaction with 5-HT neurons, we have considered the possibility that LSD can interfere with the normal maturation of the indoleamine biochemical apparatus in the brain.

METHODS

Mice of the CFW strain from Carworth Farms were maintained on Purina Lab Chow and a lighting regime of 10 hr of light and 14 hr of darkness. Animals chosen for injection were one and seven days of age. Four types of i.p. injections were made, with LSD doses of 0·5 μg/kg, 5·0 μg/kg and 50 μg/kg as well as controls injected with the carrier only. The animals were killed during the fourth hour of their daily light period when they were eight weeks of age.

Following decapitation, the brains were removed by splitting the brain case parasagitally and removing the roof. The olfactory bulbs and the hypophysis were discarded but the pineal was retained. Following removal the brains were dissected into four parts in cold 0·15 M NaCl. The cerebellum was removed first followed by the cerebral hemispheres which were individually separated by bisecting the corpus callosum, lifting the posterior portion forward and severing the connection to the rest of the brain just anterior to the optic chiasma. A mesencephalon–diencephalon fraction, which contained the pineal, was separated from a medulla-pons fraction by cutting at an oblique angle in the groove caudal to the posterior colliculus on the dorsal side and rostral to the pons on the ventral side. Each of these four parts (cerebellum, cerebral hemispheres, mesencephalon–diencephalon and medulla-pons) was blotted on filter paper and weighed to the nearest 0·1 mg after which each was assayed for one of the indoleamine pathway components.

For each pathway enzyme or intermediate, 9–10 animals were used for each dose, or control, for each injection period. All values for drugged animals were statistically compared to controls using Student *t*-test. The enzymes in the pathway were measured essentially by incubation of tissue homogenates with ^{14}C substrates and isolation of products. Tryptophan-5-hydroxylase (T5-H) assay was a modification (Kuhar *et al.*, 1971) of the CO_2 trapping method of Ichyama *et al.* (1970) using ^{14}C-tryptophan as the substrate. 5-Hydroxytryptophan decarboxylase (5-HTPD) assay involved measurement of 5-HT formed in a preparation containing ^{14}C 5-hydroxytryptophan (5-HTP) as the substrate, a MAO inhibitor (iproniazid) and pyridoxyl phosphate cofactor (Baker *et al.*, 1973a). Monoamine oxidase (MAO) was assayed by measuring 5-hydroxyindole acetic acid (5-HIAA) formed from ^{14}C 5-HT (Baker *et al.*, 1973b).

The intermediates 5-HT and 5-HIAA were measured by differential extraction and fluorometry as described by Quay (1963).

Table 1. 5-HT concentration as μg per g tissue, in mouse brain regions following LSD injection at one week postpartum measured at eight weeks of age

		Drug dose		
	Control	0·5 μg/kg	5·0 μg/kg	50·0 μg/kg
Cerebellum	*N*= 9	10	10	10
	X=0·20	0·13	0·15	0·19
	S.E.M.=0·035	0·027	0·024	0·040
Hemispheres	*N*= 10	10	10	10
	X=0·64	0·66	0·64	0·67
	S.E.M.=0·032	0·026	0·039	0·032
Mesencephalon–diencephalon	*N*= 10	10	10	10
	X=1·29	1·44	1·56*	1·57†
	S.E.M.=0·052	0·096	0·116	0·098
Medulla-pons	N= 10	9	10	10
	X=0·92	0·92	0·97	1.02
	S.E.M.=0·061	0·048	0·088	0·062

N=number of sample, *X*=mean, S.E.M.=Standard error of the mean.
* *P*=<0·05, † *P*=<0·025 using Student-*t* test and comparing to control.

RESULTS

No differences between experimentals and controls were found at either the day 1 or day 7 injection time for T5-H, 5-HTPD or 5-HIAA. 5-HT was found to be elevated in the mesencephalon-diencephalon for 7 day injected mice at both the 5·0 μg/kg and the 50·0 μg/kg dosage (Table 1). All other 5-HT values were not significantly different from controls for all doses, regions and times of exposure. MAO was elevated in the hemispheres for the 7 day injected mice at both the 5·0 μg/kg and the 50·0 μg/kg dosage (Table 2). MAO in 7 day injected mice was also elevated over controls in the medulla-pons at the 5·0 μg/kg dosage only (Table 2). All other MAO values were not significantly different from controls for all doses, regions and times of exposure.

DISCUSSION

These data do indicate that a single exposure to LSD during maturation can have a long term or permanent effect upon the brain's 5-HT biochemistry. A number of other drugs which interact with the brain's 5-HT metabolism have significant effects upon that metabolism and upon the brain in general when they are applied during the period of maturation. The 5-HT inhibitors, *p*-chlorophenylalanine and 1-phenylalanine, will cause retarded brain growth as well as behavioral defects in immature rats (Hole, 1972a; Grundt & Hole, 1971). A number of 5-HT antagonists such as cytohepladine, methysergide and BC105 (Sandoz) will increase brain 5-HT when applied to immature rats (Hole, 1972b).

It is clear from a variety of different studies on the

Table 2. MAO activity as mμ moles 5-HIAA produced per hr per mg tissue, in mouse brain regions following LSD injection at one week postpartum measured at eight weeks of age

		Drug dose		
	Control	0·5 μg/kg	5·0 μg/kg	50·0 μg/kg
Cerebellum	*N*= 11	9	9	9
	X=2·02	2·09	2·18	2·04
	S.E.M.=0·085	0·082	0·065	0·043
Hemispheres	*N*= 11	9	9	9
	X=1·16	1·19	1·28*	1·24†
	S.E.M.=0·028	0·050	0·030	0·025
Mesencephalon–diencephalon	*N*= 11	9	9	9
	X=1·76	1·79	1·89	1·84
	S.E.M.=0·055	0·068	0·049	0·056
Medulla-pons	*N*= 11	9	9	9
	X=1·78	1·86	1·97‡	1·93
	S.E.M.=0·051	0·050	0·055	0·060

N=number in sample, *X*=mean, S.E.M.=Standard error of the mean.
* *P*=<0·01, † *P*=<0·05, ‡ *P*=<0·025 using Student-*t* and comparing to control.

rat and the mouse that the 5-HT metabolism of the newborn murine brain is quite different from that of the adult (Baker & Quay, 1969; Bennett & Giarman, 1965; Eiduson, 1972). It is during the early days of postnatal life that the enzymes and intermediates of the 5-HT pathway undergo the greatest change and adjustment in their maturation toward adult status in the mouse (Baker & Hoff, 1972; Baker *et al.*, 1973a; 1973b). This pattern is generally supported by histochemical investigations of maturing 5-HT neurons in the rat (Loizou, 1972), although the mechanism of maturational control is at present a matter of disagreement (Renson, 1971). Postnatal increases in T5-H activity have been experimentally implicated as the major regulatory factor (Bennett & Giarman, 1965); however, another experimentally supported view presupposes an adequate biochemical level of activity and places primary importance upon patterns of terminal formation during neuronal outgrowth toward the adult condition (Loizou, 1972). Neither hypothesis is completely satisfactory and other factors may be involved (Baker & Quay, 1969; Eiduson, 1971; Renson, 1971).

The effects of LSD upon brain 5-HT metabolism have been extensively investigated. Following LSD exposure brain 5-HT rises, 5-HIAA falls, neither 5-HTPD nor MAO are changed and eventually all components return to the pre-drugged status (Rosecrans *et al.*, 1967; Giarman & Freedman, 1965; Tonge & Leonard, 1969). Vesicular 5-HT content was found to increase following the drug's application (Rosecrans *et al.*, 1967). This is apparently the result of decreased neuronal firing due to the unavailability of post synaptic receptor sites (Anden *et al.*, 1968; Tonge & Leonard, 1969); however there is still no clear indication whether LSD is blocking or mimicking 5-HT there (Aghajanian, 1972).

It is certainly possible that interruptive action upon this system, especially during the early postnatal phase of rapid growth, could produce abnormal differentiation of 5-HT neurons which would then be reflected as microanatomical irregularity or abnormal biochemistry in the adult. Recent histochemical mapping of the developing and maturing rat brain has demonstrated that the 5-HT neurons and their incomplete axonal extensions are laid down around the second week of prenatal development (Olson & Seiger, 1972); but the final outgrowth and establishment of terminals is accomplished during the first three weeks after birth (Loizou, 1972). In their prenatal study of 5-HT neuron formation, Olson & Seiger (1972) suggested that 5-HT may be a trophic or chemotaxic factor for nerve outgrowth during the period of morphogenesis. It is possible therefore that drug induced changes in 5-HT neurons occurring before their outgrowth is complete, could be a cause for the modified biochemistry that we see here, however proof of this kind of effect would require microanatomical studies.

None of the changes we have found are of great magnitude; they are generally in the area of 10%, which would suggest that only part of the 5-HT system was being modified by the drug. In this same vein, studies of LSD interactions with the 5-HT system indicate that effects vary depending upon the group of neurons involved (Aghajanian, 1972; Aghajanian *et al.*, 1972). Regional studies of LSD binding sites also show such variation (Farrow & Van Vunakis, 1973), and there is evidence of multiple 5-HT pools which respond differently to LSD (Shields & Eccleston, 1973). The fact that seven day LSD exposure is more effective than one day LSD exposure is probably due to the state of growth the 5-HT system is involved in at those times. In rats (Bennett & Giarman, 1965; Eiduson, 1972; Renson, 1971) and in mice (Baker & Hoff, 1972; Baker *et al.*, 1973a; 1973b) the immediate postnatal period is rather static in terms of 5-HT change; but by one week the system is going through rapid increase. Loizou (1972) has been able to show that the weeks following birth involve the outgrowth and establishment of terminals for the 5-HT neurons. We would suggest that during such a process the neurons are sensitive to LSD action and variations in maturational pattern occur.

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