 Comp. gen. Pharmac., 1972, **3**, 22–26. [Scientechnica (Publishers) Ltd.]

THE EFFECTS OF LSD ON INDOLEAMINE ENZYMES IN EMBRYONIC *XENOPUS LAEVIS*

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(*Received* 2 *June*, 1971)

ABSTRACT

1. Neurulae and post-hatched *Xenopus* embryos were exposed to LSD at a concentration of 0·06 μg. per ml. for 6 hours.
2. Monoamine oxidase (MAO) and 5-hydroxytryptophan decarboxylase (5-HTPD) activities in whole embryos were changed.
3. Hydroxyindole-*O*-methyltransferase (HIOMT) was unchanged in whole embryos.
4. MAO and 5-HTPD were unaltered in brain.
5. HIOMT was altered in brains of embryos exposed to LSD at post-hatching. The level was almost zero. It was not lowered in brains of neurulae exposed to LSD.
6. It is suggested that the result is a secondary effect of altered 5-HT ontogeny.

WHILE there is experimental support for the involvement of lysergic acid diethylamide (LSD) in developmental abnormality (Alexander, Miles, Gold, and Alexander, 1967; Auerbach and Rugowski, 1967; Gerber, 1967) the evidence is still conflicting (Warkany and Takacs, 1968; Roux, Dupuis, and Aubry, 1970). In their recent review Dishotsky, Loughman, Mogar, and Lipscomb (1971) found it difficult to make any firm assessment of the teratogenic effects of LSD. To date, investigators have concerned themselves with morphological error and germ-cell damage (Dishotsky and others, 1971). A metabolic study of LSD as an agent in abnormal development was undertaken recently with an analysis of 5-hydroxytryptamine (5-HT) in developing *Xenopus* embryos (Baker, 1971). 5-HT was selected because its interaction with LSD in adult brain has been well documented (Giarman and Freedman, 1965). *Xenopus* embryos were used for study because they have no maternal associations during development and their 5-HT metabolism has been described in some detail (Baker, 1965; 1966a; 1966b; Baker, Quay, and Axelrod, 1965).

* Supported by grants from The Health Fund of Greater Cleveland and the Cleveland State University Faculty Research Committee.

In earlier experiments an exposure of 0·06 μg. LSD per ml. aquarium water for 6 hours was found to cause a long-term depression of 5-HT levels in both whole embryos and brain (Baker, 1971). The reduction could not be explained in terms of present knowledge of LSD interaction with 5-HT in adult brain (Smythies, 1970). The enzyme studies reported here were undertaken to determine whether variations in enzymatic action could be responsible for the 5-HT changes. *In vitro* measurements were made of 5-hydroxytryptophan decarboxylase (5-HTPD), the enzyme which forms 5-HT from 5-hydroxytryptophan (5-HTP); monoamine oxidase (MAO), the enzyme which destroys 5-HT and leads to the primary pathway metabolite 5-hydroxyindole-acetic acid (5-HIAA); and hydroxyindole-*O*-methyltransferase (HIOMT), the enzyme whose product, melatonin (5-methoxy-*N*-acetyltryptamine), may be implicated in 5-HT regulation (Erspamer, 1966; Anton-Tay, Chou, Anton, and Wurtman, 1968; Fiske and Huppert, 1968; Piezzi and Wurtman, 1970).

MATERIALS AND METHODS

Embryos of *Xenopus laevis* were obtained by chorionic gonadotropin injection, and staged according to the Normal Table of Nieuwkoop and Faber (1956). After harvesting they were maintained in spring water in aerated aquaria. Groups

of embryos were exposed to LSD for 6 hours at a concentration of 0·06 μg. per ml. aquarium water (Baker, 1971). Following this they were carried through multiple rinses in spring water. Stages chosen for exposure were neurula (stage 19) and post-hatching (stage 40). Enzyme assays were carried out 5 and 7 days later at the premetamorphic stage (stage 48). Whole embryos were used in groups of 3 and brains in groups of 5.

5-HTPD activity was measured as previously described using a modification (Baker, 1966a) of the method of Snyder and Axelrod (1964). Tissues were homogenized in phosphate buffer and incubated with a monoamine oxidase inhibitor (iproniazid), pyridoxyl phosphate, and [^{14}C]5-hydroxytryptophan. Following incubation 1 ml. 0·5 *M* (*p*H 10) borate buffer and 3 ml. of a 3 : 2 mixture of *n*-butanol and chloroform were added. The tubes were stoppered, shaken, and centrifuged, and the aqueous phase was aspirated. The remaining solvent was given a 2-ml. 0·05 *M* (*p*H 10) borate buffer wash and 2·7 ml. from each tube were transferred to a series of counting vials. The vials then received 10 ml. POPOP–PPO phosphor solution and 1 ml. ethanol, and were counted in a Nuclear Chicago Unliux II. 'Heated enzyme' blanks were used for determination of blank correction values.

MAO activity was measured as previously described by a modification (Baker, 1966b) of the method of Wurtman and Axelrod (1963). Tissues were homogenized in phosphate buffer and incubated with [^{14}C]5-hydroxytryptamine. Following incubation the reaction was halted with 1 *M* HCl and the product, 5-HIAA, was extracted into diethyl ether. The ether was transferred to counting vials with POPOP–PPO solution and ethanol and was then counted.

HIOMT was measured using the method of Axelrod and co-workers (Axelrod and Weissbach, 1960; Axelrod, Wurtman, and Winget, 1964). Tissues were homogenized in the cold in phosphate buffer. From each sample 2 aliquots were transferred to 2 separate tubes. One tube received *N*-acetylserotonin (*N*-acetyl-5-hydroxytryptamine) and the other water. Both tubes received methyl-5-adenosylmethionine[methyl-^{14}C] and all tubes were incubated. After incubation all tubes received *p*H 10 borate buffer and the product, melatonin, was extracted into a 4 : 1 mixture of toluene/iso-amyl alcohol. The solvent phases from each tube were transferred to a series of counting vials with POPOP–PPO solution and ethanol and were counted. Counts per minute of vials with extracts from samples receiving no substrate (i.e. water) were subtracted from those receiving, substrate (i.e., *N*-acetylserotonin), and [^{14}C]-melatonin formed per hour was determined.

RESULTS

Tables I, II, and *III* outline the measured activities of 5-HTPD, MAO, and HIOMT respectively. For whole embryo there is a reduction of 5-HTPD activity for those embryos exposed to LSD at both neurula and post-hatching stages. In both cases the reduction is about 15 per cent. Whole-embryo MAO activities are reduced by about 15 per cent for those embryos exposed at post-hatching, but there is an increase of about 30 per cent for those embryos exposed at neurula. Whole-embryo HIOMT activities are unchanged by both exposures. The effects of LSD on brain enzyme activities contrast sharply with those of the whole embryo. 5-HTPD and MAO are unchanged after both types of exposure. HIOMT activity is unchanged after neurula exposure, but reduced essentially to zero after post-hatching exposure.

Table I.—5-Hydroxytryptophan Decarboxylase Activity* in Whole Embryos and Brains of Embryos exposed to LSD at Neurula (Stage 19) and Post-hatching (Stage 40) Stages as well as Unexposed Controls, All assayed at Premetamorphosis (Stage 48)

Tissue	Whole Embryo				Brain			
	N	$\bar{X}$	S.E.	*P*	*N*	$\bar{X}$	S.E.	*P*
Unexposed controls	9	95·0	2·57	—	9	24·8	3·49	—
Neurula exposure	9	81·0	1·32	<0·001	9	20·5	1·40	N.S.
Post-hatching exposure	9	82·2	1·59	<0·001	8	22·4	3·09	N.S.

N = Number of determinations; $\bar{X}$ = mean; S.E. = standard error of the mean; *P* (probability) derived from Student-Fisher *t*-test, comparisons made between controls and exposed groups; N.S. = not significant.

* Expressed as μμmoles 5-HT formed per structure per hour.

DISCUSSION

The LSD dose and exposure used here were selected to produce no observable morphological or behavioural abnormalities. The aim was to cause a biochemical change only, and in this respect the experiments were a success. In a previous report embryos of *Xenopus* were exposed to similar doses of LSD at various times in development and measured for 5-HT at premetamorphosis (Baker, 1971). The drug was found to produce long-term altered 5-HT levels in brain and whole embryos. The period of LSD sensitivity for brain and whole embryos differed (Baker, 1971). These enzyme studies do not serve to explain all of the 5-HT changes previously illustrated.

Whole embryos assayed at premetamorphosis following LSD exposure at the neurula stage revealed a 20 per cent reduction of 5-HT levels (Baker, 1971). These enzyme studies show that synthesis (as 5-HTPD activity), is decreased while destruction (as MAO activity) is increased. The net result of this change should be a 5-HT decrease, which has been demonstrated. Embryos exposed to the drug at post-hatching revealed no change in whole embryo 5-HT levels (Baker, 1971). The enzyme data show a reduction of both 5-HTPD and MAO; a reduction of similar magnitude. Such a harmonious enzymatic decrease could be said to have resulted in a static, unaltered 5-HT level. Whole-embryo HIOMT levels are not being affected by the drug.

Interpretation of brain enzyme data in the light of previously measured 5-HT levels is extremely difficult. It was found that neurula-exposed embryos showed no change in brain 5-HT at premetamorphosis, but

Table II.—Monoamine Oxidase Activity * in Whole Embryos and Brains of Embryos exposed to LSD at Neurula (Stage 19) and Post-hatching (Stage 40) Stages as well as Unexposed Controls, All assayed at Premetamorphosis (Stage 48)

Tissue	Whole Embryo				Brain			
	N	$\bar{X}$	S.E.	P	N	$\bar{X}$	S.E.	P
Unexposed control	9	662·7	24·42	—	9	181·4	6·18	—
Neurula exposure	9	876·8	20·51	<0·001	9	172·5	6·62	N.S.
Post-hatching exposure	9	547·9	21·46	<0·005	9	174·3	6·23	N.S.

N = Number of determinations; $\bar{X}$ = mean; S.E. = standard error of the mean; P (probability) derived from Student-Fisher t-test, comparisons made between controls and exposed groups; N.S. = not significant.

* Expressed as μμmoles 5-HIAA formed per structure per hour.

Table III.—Hydroxyindole-*O*-methyltransferase Activity * in Whole Embryos and Brains of Embryos exposed to LSD at Neurula (Stage 19) and Post-hatching (Stage 40) Stages as well as Unexposed Controls, All assayed at Premetamorphosis (Stage 48)

Tissue	Whole Embryo				Brain			
	N	$\bar{X}$	S.E.	P	N	$\bar{X}$	S.E.	P
Unexposed controls	7	2·8	0·25	—	6	0·5	0·13	—
Neurula exposure	7	3·0	0·18	N.S.	7	0·3	0·09	N.S.
Post-hatching exposure	7	2·7	0·29	N.S.	7	−0·1	0·11	<0·005

N = Number of determinations; $\bar{X}$ = mean; S.E. = standard error of the mean; P (probability) derived from Student-Fisher t-test, comparisons made between controls and exposed groups; N.S. = not significant.

* Expressed as μμmoles melatonin formed per structure per hour.

post-hatching-exposed embryos had a 70 per cent reduction (Baker, 1971). Here, enzyme activities for 5-HT synthesis and destruction are unchanged under similar experimental conditions. The effect of LSD upon brain 5-HT cannot, then, be explained in terms of altered activity for these two enzymes. The reduction of HIOMT after post-hatching exposure is interesting, since this part of the indoleamine pathway has not been previously assessed in relation to LSD. Whether the reduced 5-HT levels in brains of embryos exposed at post-hatching are the result of HIOMT variation is not clear at this time.

These studies of LSD action on embryonic 5-HT metabolism can be generalized to some extent. Brain and whole-embryo 5-HT development is being affected; the action is not uniform; there are different times of sensitivity and dissimilar effects upon the enzymes involved. Measurements of 5-HTPD, MAO, and HIOMT in normal development show a difference in pattern between whole embryos and brain (Baker, 1966a; 1966b; Baker and others, 1965). Whatever regulatory mechanisms are responsible for the ontogeny of 5-HT metabolism, they appear to be variable by structure and time in development, and this is being reflected in these LSD experiments.

Most studies of LSD action have been upon adult mammalian brain (Giarman and Freedman, 1965). The drug causes an elevation of 5-HT and a reduction of 5-HIAA with a return to normal levels, all within an hour (Rosecrans, Lovell, and Freedman, 1967). During these events there is no change in 5-HTPD or MAO activity. There is, however, an increase in subcellular vesicular binding of 5-HT (Diaz, Ngai, and Costa, 1968). This enhanced vesicular retention is considered to be the result of reduced or inhibited neuronal firing (Foote, Sheard, and Aghajanian, 1969).

The lack of LSD effect upon 5-HTPD and MAO in developing *Xenopus* brain conforms to data on adult mammalian brain; but the direction and duration of the 5-HT change is different. The extent to which the two systems can be compared is unclear. Since LSD is having an effect on the development of the 5-HT pathway, one would like to know if 5-HT alone is associated with developmental error. Some reports are available. In rats and mice 5-HT injected into the maternal system has caused abnormal development of the foetal brain (Poulson, Robson, and Sullivan, 1963; Reddy, Adams, and Baird, 1963; Seller, 1964). In *Xenopus* 5-HT has been elevated by rearing embryos in an MAO inhibitor (iproniazid) and abnormal brain development has occurred (Baker and Orr, 1971). The possibility then exists that LSD is causing error indirectly through altered 5-HT metabolism. The data reported here and previously (Baker, 1971) make such an indirect mechanism worthy of further investigation.

SUMMARY

Xenopus embryos were exposed to LSD at concentrations of 0·06 µg. per ml. aquarium water for 6 hours at neurula and post-hatching stages. After developing to pre-metamorphic stage whole embryos and brains were assayed for monoamine oxidase, 5-hydroxytryptophan decarboxylase, and hydroxyindole-*O*-methyltransferase activities. MAO and 5-HTPD activities in whole embryo were changed, but the changes were in keeping with previously described 5-hydroxytryptamine changes in LSD-treated *Xenopus* embryos. HIOMT was unchanged in whole embryo. MAO and 5-HTPD were unaltered in brain for both LSD exposure groups. HIOMT was unaltered in brains of neurula-exposed embryos but was reduced to essentially zero in brain of embryos exposed at post-hatching. Brain enzyme changes could not be used to explain reduced levels of 5-HT found in previous LSD experiments.

The differences between LSD effects on adult mammalian brain chemistry and developing *Xenopus* are discussed and the suggestion is made that LSD action on embryonic development may be the result of a secondary effect of altered 5-HT ontogeny.

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Key Word Index: LSD, monoamine oxidase, 5-hydroxytryptophan decarboxylase (5-HTPD), hydroxyindole-*O*-methyltransferase (HIOMT), *Xenopus laevis*, brain.