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INCREASE IN BRAIN SEROTONIN IN EXPERIMENTAL PORPHYRIA

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Abstract—Three chemically unrelated compounds, a 5βH steroid, a barbiturate, and a substituted collidine, induce δ-aminolevulinic acid synthetase in the livers and lead to increases in the concentration of serotonin in the brains of 13-day-old chick embryos. The temporal relationship between these two events suggests that they are independent effects of the drugs. The bearing of these findings on acute intermittent porphyria is discussed.

ACUTE intermittent porphyria is a rare inherited disease. Biochemically it is characterized by an increase in the urinary excretion of δ-aminolevulinic acid (ALA) and porphobilinogen.[1] These biochemical features can be produced in experimental animals by a variety of drugs,[2,3] thereby providing a model for the human disease. The elevated synthesis of these porphyrin precursors is a consequence of an increase in the activity of hepatic ALA synthetase, the rate-limiting enzyme in heme biosynthesis,[3] in experimental porphyria[3,4] and acute intermittent porphyria.[5] In humans the disease does not become manifest until after puberty, and in some women attacks have been related to the menstrual cycle.[6] These observations implicate the sex steroids. Therefore, it was of great interest when Granick *et al.* showed that a variety of natural steroids which are degradation products of the gonadal steroids are inducers of ALA synthetase in embryonic chick liver *in vitro*[7] and *in vivo*.[8]

Clinically acute intermittent porphyria is manifested by neurological disorders and is sometimes accompanied by depression, confusion or visual hallucinations.[6] The relationship between the aberration in heme synthesis in the liver and the nervous system is enigmatic.

A growing body of evidence implicates disturbances in serotonin metabolism in mental disorders. The present investigation was undertaken to determine whether serotonin metabolism in the brain is affected in experimental porphyria produced in chick embryos by three chemically dissimilar compounds, a 5βH steroid, a barbiturate, and a substituted collidine.

MATERIALS AND METHODS

Fertilized White Leghorn eggs were obtained from a local hatchery and incubated at 38° in a forced draft incubator. At various times on the twelfth day of development a hole was made in the blunt end of the egg and the drug of interest in 0·5 ml of saline was injected into the air space. The drugs studied and their dosages were 5β-pregnane-3α, 17α-diol-11, 20-dione, 5 mg in suspension, pentobarbital, 1 mg in solution, and

1,3-dicarbethoxy-1,4-dihydrocollidine (DDC), 40 mg in suspension. To facilitate uptake of the drug, a small tear was made in the inner shell membrane. The hole was closed with cellophane tape.

On the thirteenth day of development brains from six to seven embryos were pooled and separated into two parts, the cerebral hemispheres and the remainder. The serotonin concentration in these samples was determined by the fluorometric method of Snyder *et al.*,[10] using a Corning filter 7-51 for the primary filter and a secondary filter compounded from Corning filters 3-71 and 4-72. Since porphyrins are not extracted into butanol as is serotonin, the possibility that porphyrins might interfere with the fluorescence of serotonin in this assay was precluded.

Six to seven livers from other 13-day-old embryos were pooled and the activity of ALA synthetase was assayed in homogenates by the method of Marver *et al.*[11]

RESULTS

The concentration of brain serotonin and the activity of hepatic ALA synthetase in embryos injected with 0·5 ml of saline were not different from those of untreated embryos.

Pregnanedioldione led to an increase in the activity of ALA synthetase in the liver with the greatest enhancement occurring at 12 hr (Table 1). At 6 hr, when the induction of ALA synthetase was beginning to be apparent, a more than 2-fold increase in the concentration of serotonin in the cerebral hemispheres and the other parts of the brain occurred. By 12 hr the concentration of serotonin in the cerebral and noncerebral parts of the brain dropped to near normal values although they remained slightly elevated even after 24 hr.

TABLE 1. EFFECTS OF 5 mg OF 5β-PREGNANE-3α, 17α-DIOL-11, 20-DIONE ON BRAIN SEROTONIN AND LIVER δ-AMINOLEVULINIC ACID SYNTHETASE

Time after injection (hr)	Serotonin (ng/g)* Cerebrum	Remainder	nmole ALA/g/hr* Liver
0	46 ± 3	76 ± 5	0
6	129 ± 31†	200 ± 50†	11 ± 1‡
12	81 ± 7†	105 ± 15	17 ± 1‡
24	69 ± 6†	100 ± 8	11 ± 2‡

* Each value is the mean ± standard deviation of at least four experiments.
† Different from zero time with P = 0·014 according to Mann–Whitney U test.
‡ Different from zero time with P = 0·003 according to Mann–Whitney U test.

Pentobarbital also led to an increase in ALA synthetase activity in the liver with a maximum being reached in 12 hr (Table 2). Again after 6 hr of exposure to the drug, the concentration of serotonin in the cerebral hemispheres and the other parts of the brain was increased. The magnitude of this response was highly variable. Within 12 hr the concentration of serotonin returned to near normal levels in both parts of the brain.

TABLE 2. EFFECTS OF 1 mg OF PENTOBARBITAL ON BRAIN SEROTONIN AND LIVER δ-AMINOLEVULINIC ACID SYNTHETASE

Time after injection (hr)	Serotonin (ng/g)* Cerebrum	Remainder	nmoles ALA/g/hr* Liver
0	46 ± 3	76 ± 5	0
6	226 ± 108†	160 ± 27	16 ± 1‡
12	65 ± 1†	101 ± 6†	24 ± 2‡
24	93 ± 20	117 ± 21	25 ± 3‡

* Each value is the mean ± standard deviation of at least four experiments.
† Different from zero time with $P \leq 0.014$ according to Mann–Whitney U test.
‡ Different from zero time with $P = 0.003$ according to Mann–Whitney U test.

DDC like pregnanedioldione and pentobarbital produced an elevation in the activity of ALA synthetase in the liver with the highest level being attained 12 hr after the drug was given (Table 3). At 6 hr the concentration of serotonin in the cerebral hemispheres and the remainder of the brain was increased 128 and 67 per cent respectively. However, during the next 6 hr, while the concentration of serotonin in the noncerebral part of the brain declined, it continued to rise in the cerebral hemispheres reaching values three to four times normal. By 24 hr it too fell to near normal.

DISCUSSION

Pregnanedioldione, pentobarbital, and DDC, although unrelated in chemical structure, have in common the ability to induce ALA synthetase in embryonic chick liver. They also share the property of modifying serotonin metabolism in the brain so as to bring about significant increases in the concentration of serotonin. Before the induction of ALA synthetase has reached a maximum, the concentration of serotonin in the cerebral hemispheres and the other parts of the brain rises. Then, while the ALA

TABLE 3. EFFECTS OF 1,3-DICARBETHOXY-1,4-DIHYDROCOLLIDINE ON BRAIN SEROTONIN AND LIVER δ-AMINOLEVULINIC ACID SYNTHETASE

Time after injection (hr)	Serotonin (ng/g)* Cerebrum	Remainder	nmoles ALA/g/hr* Liver
0	46 ± 3	76 ± 5	0
6	105 ± 11†	127 ± 4†	12 ± 3‡
12	176 ± 48†	104 ± 11	30 ± 7‡
24	67 ± 7†	116 ± 27	20 ± 4‡

* Each value is the mean ± standard deviation of at least four experiments.
† Different from zero time with $P = 0.014$ according to Mann–Whitney U test.
‡ Different from zero time with $P = 0.003$ according to Mann–Whitney U test.

synthetase activity climbs to a maximum, the serotonin concentration in the brain falls to near normal levels. Hence, the changes observed in brain serotonin cannot be a result of enhanced rates of porphyrin synthesis in the liver. Using embryonic chick liver cells in primary culture, Granick[12] has shown that the drugs which induce ALA synthetase act directly on the liver cells. Thus, it is likely that the induction of ALA synthetase in the liver and altered serotonin metabolism in the brain are independent effects of the porphyrinogenic drugs.

In previous investigations the induction of ALA synthetase in the livers of intact chick embryos by pregnanedioldione[8] and DDC[13] yielded much higher values than those found in this study. Apparently the responsiveness of chick embryos to these substances depends on the flock from which they come.

A number of indolealkylamines and related compounds affect mental activity, including the potent hallucinogen LSD. Freedman[14] has shown that LSD produces an increase in the concentration of serotonin in the brain. In the presence of inhibitors of serotonin synthesis, LSD reduces the rate of depletion of serotonin.[15] This observation and the demonstration that the transmission of nervous impulses is required for the depletion of serotonin[16] support the conclusion that LSD interferes with transmission of serotonergic neurons.

The present study provides no information on the mechanism by which serotonin metabolism is altered in experimental porphyria. However, it does suggest a possible link between the induction of hepatic ALA synthetase and a predisposition to the neuropsychiatric symptoms of acute intermittent porphyria. A metabolite, such as a steroid of the 5βH type, might induce ALA synthetase in the liver and independently block the transmission of nervous impulses in serotonergic neurons, thereby predisposing the individual to further neurochemical events which produce visual hallucinations or other neuropsychiatric manifestations.

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