NOTE

Inhibitory Effect of 5-Hydroxytryptophane on the Induction of Persistent Estrus by Androgen in the Rat

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Synopsis

Effects of monoamines or its precursors on the induction of persistent estrus by testosterone propionate (T.P.) were studied in Wistar strain female rats.

Daily treatment with 5-hydroxytryptophane (5-HTP) for the first 10 days of life delayed the occurrence of persistent estrus in rats given T.P. at 4 days of age. The onset of persistent estrus occurred in this group of animals at 67.1 ± 2.5 days of age compared with 45.1 ± 1.4 days of age for the saline treated control group. The role of hypothalamic monoamines in contributing to the induction of persistent estrus by T.P. treatment of neonatal rats is discussed.

Androgen administration during the first 10 postnatal days in female rats interferes with the normal differentiation of the anterior hypothalamus leading to persistent vaginal cornification in the adult (see for review: Takewaki, 1962; Barraclough, 1966). However, the effects of neonatal treatment with androgen vary considerably in degree, according to the dose of androgen administered (Gorski, 1963; Kikuyama, 1963), the age at injection (Kikuyama, 1962; Gorski, 1966) and the duration of the period of administration (Arai, 1964; Kawashima, 1965). Previously, Gorski (1966) had concluded that an injection of 10 μ g testosterone propionate (T. P.) on day 4 or 5 was the minimum effective dose for androgenization of the female rat.

It has been reported that the persistent-estrus inductive influence of sex steroids was markedly attenuated or abolished by reserpine (Kikuyama, 1961; Arai and Gorski, 1968; Shirama *et al.*, 1974). In

recent years, considerable attention to this phenomenon has been focused on the possible involvement of brain serotonin (Ladosky and Gaziri, 1970; Campos and Ladosky, 1972; Guilian *et al.*, 1973; Hardin, 1973; Hyypä and Cardinali, 1973).

The present study was undertaken to determine whether precursor of serotonin known to enter the brain and to increase brain serotonin concentration (Fernstrom and Wurtman, 1971) could alter the induction of persistent estrus by androgen injection in the rat.

Materials and Methods

Wistar female rats were bred in our colony and the day of delivery counted as day 0 for the young. All litters were reduced to eight pups. If a litter did not contain eight pups, female pups from other litters of the same age were added to bring the total up to eight.

Thirty-seven out of 74 female pups were divided into 5 groups having 7-8 pups, respectively. Each group was injected subcutaneously with saline only (Group a), 3-hydroxytyramine=dopamine (Group b),

serotonin creatine sulfate=5-hydroxytryptamine creatine sulfate=5-HT (Group c), L-3, 4-dihydroxyphenylalanine=L-dopa (Group d) and 5-hydroxytryptophane=5-HTP (Group e) dissolved in 0.02 ml of physiological saline for 10 successive days starting with the day of birth. Daily dose was 100 μ g, respectively. The other thirty-seven were given a single subcutaneous injection of testosterone propionate=T.P. (10 μ g in 0.02 ml of sesame oil) at 4 days of age and were divided into five groups. Each group was injected with saline (Group 1), dopamine (Group 2), 5-HT (Group 3), L-dopa (Group 4) and 5-HTP (Group 5). These treatments were made as described above. All animals were kept in a temperature-controlled, artificially illuminated room with a light:dark cycle of 12:12 hr. and allowed access to laboratory chow and water *ad libitum*. The time of vaginal opening and first estrus were noted, and vaginal smears were examined every day until sacrifice at 100 days of age. At sacrifice, ovaries, uteri, hypophysis and adrenals were removed, weighed and processed for histological examination.

Statistical evaluation was performed using the Student's *t* test.

Results

The data in Table 1 show that subcutaneous injections of the monoamines or their precursors for ten consecutive days starting

with the day of birth had no effect on the time of vaginal opening. In 5-HT-treated rats, the day of the first vaginal cornification, index of puberty, was observed as late as saline-treated control rats (5-HT-treated rats, 34.1 ± 0.8 days vs saline-treated rats, 32.1 ± 0.5 days, $P < 0.05$). All females receiving saline only, dopamine, 5-HT and L-dopa exhibited regular-4-day estrous cycles (Fig. 1). The ovaries of those groups had both follicles and well-developed corpora lutea, and were not significantly different in structure and in weight between them (Table 2). The other group treated with 5-HTP only showed prolonged estrus especially for the early experimental period. The appearance of ovaries in this group was similar to that of the above groups.

All of eight saline-treated rats which had received an injection of androgen at 4 days of age showed continued vaginal cornification after cyclic or prolonged estrus took place for 38–50 days of age (45.1 ± 1.4 days) (Fig. 2). The day of vaginal opening in this group was observed at 13–18

Table 1. Effects of monoamines or their precursors on the day of vaginal opening and first estrus in the rats.

Groups	Injection	No. of rats	Age at vaginal opening (days)	Age at first estrus (days)
a	Saline	8	$32.1 \pm 0.5^{1)}$	32.1 ± 0.5
b	Dopamine	7	33.8 ± 0.9	34.3 ± 0.8
c	5-HT	7	$34.1 \pm 0.6^{*2)}$	33.9 ± 1.0
d	L-Dopa	7	33.6 ± 0.8	33.9 ± 1.0
e	5-HTP	7	31.1 ± 0.6	31.1 ± 0.6

1) Mean value $\pm$ standard error.

2) * $P < 0.05$: Significantly different as compared with Group a.

Table 2. Body weights and organ weights per 100 g body weight in saline-, dopamine-, 5-HT-, L-dopa- and 5-HTP-treated rats.

Groups	Injection	No. of rats	Body weight (g)	Ovaries	Uteri	Hypophysis	Adrenals
				(mg per 100 g body weight)			
a	Saline	8	$293 \pm 4^{1)}$	27.31 ± 0.84	148.4 ± 9.1	3.98 ± 0.41	28.54 ± 0.91
b	Dopamine	7	300 ± 2	27.73 ± 0.78	153.2 ± 12.5	4.01 ± 0.35	31.52 ± 0.44
c	5-HT	7	289 ± 4	26.29 ± 1.26	158.4 ± 20.9	3.69 ± 0.26	28.91 ± 1.36
d	L-Dopa	7	298 ± 6	28.73 ± 1.75	151.4 ± 23.3	3.50 ± 0.11	31.76 ± 1.38
e	5-HTP	7	301 ± 7	27.64 ± 0.54	129.4 ± 4.3	3.80 ± 0.26	30.65 ± 0.33

1) Mean value $\pm$ standard error.

days of age (15.8 ± 1.4 days) and the day of the first estrus occurred at 31–36 days of age (32.9 ± 0.7 days) (Table 3).

In dopamine-, 5-HT- and L-dopa-treated rats which had been injected with androgen at 4 days of age, although two animals of each group exhibited persistent estrus starting with the day of the first estrus, the day

of persistent estrus in those groups was not significantly different as compared with saline-treated control rats. On the other hand, all of 7 5-HTP-treated animals which had received an androgen injection at 4 days of age showed cyclic or prolonged estrus before continued vaginal cornification took place for 57–73 days of age (67.1 ± 2.5

Fig. 1. Vaginal smear records in some monoamines or their precursors-treated rats. The thin baseline of the figures corresponds to non-estrous stages which contain proestrus, metestrus and diestrus and thick top to perfect estrus. Open circles show the day of vaginal opening.

Fig. 2. Effects of early injections of some monoamines or their precursors on estrous cycles in rats androgenized at 4 days of age. The thin baseline of the figures corresponds to non-estrous stages which contain proestrus, metestrus and diestrus and thick top to perfect estrus. Closed circles indicate the day of first estrus.

Table 3. Effects of monoamines or their precursors on the day of vaginal opening, first estrus and persistent estrus in rats androgenized at 4 days of age.

Groups	Injections	No. of rats	Age at vaginal opening (days)	Age at first estrus (days)	Age at onset of persistent estrus (days)
1	T.P. on day 4 + Saline	8	$15.8 \pm 2.4^{1)}$	32.9 ± 0.7	45.1 ± 1.4
2	T.P. on day 4 + Dopamine	7	15.9 ± 3.1	32.6 ± 0.7	43.1 ± 4.4
3	T.P. on day 4 + 5-HT	7	16.3 ± 2.4	31.3 ± 0.4	43.4 ± 3.3
4	T.P. on day 4 + L-Dopa	8	16.5 ± 1.9	32.3 ± 0.5	38.1 ± 2.0
5	T.P. on day 4 + 5-HTP	7	17.4 ± 2.1	32.6 ± 0.8	$67.1 \pm 2.5^{*2)}$

1) Mean value $\pm$ standard error.

2) * $P < 0.01$: Significantly different as compared with Group 1.

Table 4. Effects of early monoamines or their precursors treatments on average weights of some organs (mg) per 100 g body weight in rats androgenized at 4 days of age.

Groups	Injections	No. of rats	Body weight (g)	Ovaries	Uteri	Hypophysis	Adrenals
				(mg per 100 g body weight)			
1	T.P. on day 4 + Saline	8	289±6 ¹⁾	14.95±0.73	143.3± 9.4	5.50±0.19	32.11±0.97
2	T.P. on day 4 + Dopamine	7	301±5	17.38±1.21	159.3±18.8	4.69±0.25 ^{**2)}	34.75±0.25
3	T.P. on day 4 + 5-HT	7	288±7	15.90±1.18	122.6± 4.8	4.01±0.29 ^{**}	33.88±2.88
4	T.P. on day 4 + L-Dopa	8	279±7	16.97±0.44	132.4± 5.3	5.57±0.48	38.15±0.68 ^{**}
5	T.P. on day 4 + 5-HTP	7	302±5	20.37±1.30 ^{**}	176.1± 7.3	4.94±0.14	37.64±0.64 ^{**}

1) Mean value±standard error.

2) *P<0.05, **P<0.01: Significantly different as compared with Group 1.

days, Group 1 vs Group 5, $P<0.01$) (Table 3 and Fig. 2). These rats were also significantly different in ovarian weight as compared with controls (Group 1, 14.95 ± 0.73 mg vs Group 5, 20.37 ± 1.30 mg, $P<0.01$) (Table 4).

All of 6 groups receiving an androgen injection at 4 days of age contained follicles of varying sizes but lacked corpora lutea and were not significantly different in structure between them.

Discussion

The present observations indicate that subcutaneous injections of 5-HTP, the immediate serotonin (5-HT) precursor, inhibit the androgenization of the hypothalamic centre of ovulatory control although the exact mechanism is unknown. Previously, Vaughan *et al.* (1969) reported that 5-HT interrupted persistent estrus by androgen treatment. However, 5-HT did not inhibit the action of testosterone propionate (T. P.) on the induction of persistent estrus at present. Inasmuch as blood-brain barrier in the neonatal rat brain was not well developed, 5-HT readily passed through it (Karki *et al.*, 1962). Its effect on the induction of persistent estrus by androgen

may be limited entirely to the dose of 5-HT and to the age of injections.

Recently, it was found that the injections of monoamine oxidase inhibitor (MAOI), which increased brain 5-HT concentration (Shore, 1962), into androgenized baby rats could result in accelerated onset of persistent estrus (Shirama *et al.*, in contribution). On the other hand, reserpine, an agent which depletes brain 5-HT (Shore *et al.*, 1955), was capable of abolishing the effect of sex steroid on the anterior hypophysis in baby rats (Kikuyama, 1961; Arai and Gorski, 1968; Shirama *et al.*, 1974). Thus, although the injection of androgen into neonatal females results in reduced brain 5-HT concentration (Guilian *et al.*, 1973), 5-HTP effect on the responsiveness to T. P. may not involve 5-HT levels in the neonatal rat brain.

Reserpine causes profound changes in 5-HT metabolism. It impairs 5-HT storage (Brodie *et al.*, 1957), facilitates 5-HT availability to monoamine oxidase (MAO), and, consequently, accelerates brain turnover rate (Tozer *et al.*, 1966). If those mechanisms were available to regulate biosynthesis to tryptophane hydroxylase, they might be activated during the persistent action of reserpine on 5-HT storage process. Zivkovic *et al.* (1973) found that reserpine

injections produce a long-term increase of tryptophane hydroxylase in the rat central nervous system. Besides, the activity of tryptophane hydroxylase is regulated by endoproduct inhibition (Neff and Costa, 1966; Nagatsu *et al.*, 1964). Therefore, it seems very likely that the decrease of susceptibility to androgen by 5-HTP injections results in the increase of tryptophane hydroxylase and/or MAO activity. Thus, in the present results, this effect of 5-HTP on the induction of persistent estrus by T. P. injection may suggest an involvement of the maturation of serotonergic pathway in the site(s) of androgenization of the brain. However, further studies will be needed to get a definite conclusion.

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