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Reinitiation of Estrous Cycles in Light-Induced Constant Estrous Female Rats by Drugs (38233)

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Prolonged exposure to continuous light induces an anovulatory persistent estrous state in the female rat (1–3). Pituitaries of such rats have been reported to contain less LH (4, 5) while Negro-Vilar (6) observed no change in pituitary FSH but significantly more hypothalamic FSH–RF activity. Recently we noted that female rats in light-induced constant estrus showed increases in serum FSH and prolactin whereas LH remained at basal level (unpublished observations).

Pituitary release of prolactin, LH and FSH is controlled by hypothalamic hypophysiotropic factors, which in turn are regulated at least in part by hypothalamic monoamines (7–10). Since we recently observed reinitiation of estrous cycles in old constant estrous rats by administering L-dopa, epinephrine or iproniazid, thereby correcting a presumed hypothalamic deficiency of catecholamines (11), it was of interest to see whether estrous cycles could be re-established in light-induced constant estrous rats by pharmacologic manipulation of hypothalamic monoamines.

Materials and Methods. Daily vaginal smears were taken from 3–4 mo old Sprague–Dawley female rats (Spartan Research Animals, Inc., Haslett, MI) exposed to constant illumination. Overhead fluorescent lighting was supplemented by 100-w white-light bulbs placed about 5 feet above transparent plastic cages. All rats were maintained on a diet of Wayne Lab Blox pellets (Allied Mills, Chicago, IL) and tap water was supplied *ad libitum*. Only animals exhibiting 10 successive days of persistent vaginal estrus after 50–60 days exposure to continuous light were selected for this study.

On the 10th day of constant vaginal estrus, the female rats were randomly divided into groups and injected daily with drugs as shown in Table I. All drugs were injected sc in .85% NaCl or in a corn oil suspension. The drugs were administered at 10 AM daily except for L-dopa and methyldopa which were given sc 2 times daily at 10 AM and 10 PM, and PCA which was injected ip every 5th day at 10 AM. Daily monitoring of vaginal cycles continued throughout the treatment period.

Since the only treatment that was successful in reestablishing normal estrous cycles was the combination of L-dopa and PCA, this combination was tested again in 30 similar light-induced constant estrous rats. Serum LH concentration was determined in animals in this group that exhibited 3 consecutive estrous cycles, by bleeding via cardiac puncture under light ether anesthesia at 2-hr intervals, from 8 AM–8 PM on proestrous day of the 3rd cycle. Ovulation was confirmed the next morning by removing the oviducts and ovaries and examining under the dissecting microscope for the presence of tubal ova and for new corpora lutea. The serum was separated and stored at −20° until assayed for LH by the radioimmunoassay method of Niswender

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TABLE I. Drugs Used in Attempt to Reinitiate Estrous Cycles in Rats Under Constant Light.

Treatment + no. of rats	Dose/KG BW	Purpose
Controls (10)	Vehicle Only	—
L-Dopa (10)	100 MG	inc. CA[a]
α-M-P-Tyrosine (AMPT) (10)	200 MG	dec. CA
5-Hydroxytryptophan (5-HTP) (10)	100 MG	inc. 5HT
para-Chloroamphetamine (PCA) (10)	3.5 MG	dec. 5HT
L-Dopa + PCA (15)	100 MG + 3.5 MG	inc. CA, dec. 5-HT
AMPT + 5-HTP (15)	200 MG + 100 MG	dec. CA, inc. 5-HT
AMPT + PCA (15)	200 MG + 3.5 MG	dec. CA, dec. 5-HT
L-Dopa + 5-HTP (15)	100 MG + 100 MG	inc. CA, inc. 5-HT
Reserpine (5)	10 MG	dec. CA, dec. 5-HT
Methyldopa (5)	80 MG	dec. CA, dec. 5-HT
Iproniazid phosphate (5)	100 MG	inc. CA, inc. 5-HT

[a] CA = Catecholamine, 5-HT = 5-Hydroxytryptamine (serotonin) inc. = increase, dec. = decrease.

et al. (12), using NIAMD-LH-RP-1 as the reference standard.[3]

Results. Only the combination of L-dopa and PCA initiated cycling, with 9 of 15 animals in this group showing at least 2 consecutive estrous cycles. None of the other drug treatments resulted in estrous cycles. When the combination of L-dopa and PCA was tested again in 30 similar constant estrous rats, 22 exhibited 3 consecutive cycles. Thus of the total of 45 rats treated with the PCA and L-dopa combination, estrous cycles were induced in 31. Of these, 28 began their cycles within 2 days after initiation of treatment and 24 were judged to cycle every 5 days. The other 4 cycled every 4 or 5 days.

Of these 22 rats that cycled in the 2nd experiment, 17 had ova and fresh corpora lutea upon examination at autopsy, and all 17 had serum LH peaks on proestrous day (Table II). The 5 rats that failed to ovulate (nos. 2, 7, 11, 18, 19) did not show LH peaks; however, it is possible that these animals had LH peaks and ovulated at a time not monitored in this study. It can be seen that the LH elevations occurred over a wide range of time, from 10 AM to 6 PM. Only 1 rat (no. 17) showed a surge in serum LH at 10 AM and exhibited a second smaller rise at 4 PM. Two rats showed a peak elevation in serum LH at 12 Noon, 9 at 2 PM, 3 at 4 PM and 2 at 6 PM.

Discussion. These results indicate that a combination of PCA and L-dopa can restore vaginal estrous cycles in persistent estrous rats while still under constant illumination. PCA selectively reduces 5-HT stores (13–15) whereas L-dopa increases catecholamine activity (16). The failure of either PCA or L-dopa alone to induce cycling suggests a dual blockade of ovulation in these females. Both increased central 5-HT (17–19) as well as decreased central catecholamines (20) have been reported to inhibit ovulation. It appears, therefore, that a balance between these monoamines operates to regulate ovulation and cycling, and that constant illumination disturbs this balance.

A preliminary report by Miller *et al.* (15) suggests that a single injection of 3.5 mg/kg PCA reduces hypothalamic 5-HT for at least 8 days. In the present study, the schedule of injecting PCA every 5th day was chosen to insure a continuous reduction of 5-HT levels. It is possible, however, that injection of the combination of PCA and L-dopa stimulated ovulation as a result of stress and the cycle was then maintained by L-dopa alone until the 2 drugs were ad-

[3] Anti-ovine LH serum was generously provided by Dr. G. Niswender (Colorado State University, Fort Collins, CO), and purified LH for radioiodination was kindly provided by Dr. Leo Reichert (Emory University, Atlanta, GA).

TABLE II. Serum LH (ng/ml NIAMD-LH-RP-1) on Proestrous Day in Rats Exhibiting Estrous Cycles Under Constant Light and Given Daily Injections of L-Dopa and PCA.

Rat No.	8 AM	10 AM	12 N	2 PM	4 PM	6 PM	8 PM
1	18	54	29	25	*432*	36	31
2	19	28	37	35	33	28	36
3	58	9	14	28	618	*1296*	24
4	12	10	17	17	*612*	37	64
5	26	22	16	*390*	150	25	44
6	23	28	34	*443*	18	19	42
7	14	16	29	34	15	32	33
8	25	22	596	*1408*	39	19	39
9	23	44	22	*446*	19	5	12
10	29	31	*451*	29	29	26	29
11	19	11	12	18	31	19	29
12	16	23	25	*356*	29	37	64
13	45	220	*394*	40	39	48	55
14	12	10	18	21	54	*908*	106
15	22	17	447	*518*	34	21	27
16	42	34	52	*300*	25	52	150
17	19	*441*	17	14	333	21	32
18	14	12	19	16	36	19	22
19	36	33	30	39	25	33	12
20	42	22	36	*296*	22	33	19
21	12	34	42	20	*390*	28	22
22	27	34	148	*530*	459	33	29

ministered again on the next 5th day. This possibility is suggested by the observation that most animals exhibited 5-day cycles. Brown-Grant (21) reported that stressful stimuli can induce ovulation in female rats exposed to constant light.

It is of interest that the LH peaks in the rats induced to cycle with the PCA and L-dopa combination appeared randomly on the day of proestrus. By contrast, normal cycling female rats show a serum LH peak on the late afternoon of proestrus (22–23), and our own Sprague-Dawley rats show a LH peak between 5–7 PM. Since blood samples were collected while the animals were under constant light, the randomness of the LH surges may reflect individual rhythms in the LH releasing apparatus.

Daily rhythms of serotonin have been reported in the pineal, cerebral cortex, brainstem and hypothalamus of the rat (24–25). In each tissue the highest level of 5-HT occurred during the light period and was lowest during darkness. The pineal serotonin rhythm could be inverted by reversing the lighting regimen (26) and the nocturnal decline was prevented by exposure to continuous lighting (27). It appears, therefore, that constant illumination may alter the 5-HT rhythms in several brain regions, including the hypothalamus. Direct measurements of 5-HT as well as catecholamines in the hypothalamus after constant light exposure remain to be determined. However, the present results suggest that light-induced constant estrous is accompanied by high 5-HT and low catecholamines in the hypothalamus. Pharmacological reversal of these amine levels stimulated ovulation as well as cycling.

In a recent report, Takahashi *et al.* (28) claimed that ovulation and cycling could be produced in light-induced persistent estrous rats by administration of serotonin or 5-HTP. However, he also reported that ether stress induced ovulation in these rats and there was no clear indication that regular cycling actually occurred. Furthermore, other evidence indicates that serotonin is inhibitory to ovulation (18–20), and our present study clearly shows that 5-HTP, the precursor of serotonin, has no ability to

initiate cycling in light-induced constant estrous rats.

Summary. Light induced constant estrous female rats were injected daily with central acting drugs that either increased or decreased brain catecholamines or serotonin or altered levels of both amines. Only the combination of L-dopa and p-chloroamphetamine (PCA) was effective in reinitiating regular estrous cycles in the rats while still under constant light. Nine of 15 rats so treated with PCA and L-dopa in the first experiment showed at least 2 consecutive cycles, and 22 of 30 rats in a second experiment showed 3 consecutive cycles. Serum LH was observed from 10 AM to 6 PM on the day of proestrus in the 22 cycling rats of the second experiment. Rats showing LH peaks ovulated by the next day. These results suggest that constant light induces a deficiency of catecholamines and an excess of serotonin in the hypothalamus, thereby preventing normal LH release.

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