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Catecholaminergic-Serotonergic Balance in the CNS and Reproductive Cycling in Aging Rats

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COOPER, R. L., M. C. McNAMARA AND M. LINNOILA. *Catecholaminergic-serotonergic balance in the CNS and reproductive cycling in aging rats.* NEUROBIOL AGING 7(1) 9–15, 1986.—Treatment with the serotonin (5-HT) reuptake inhibitor zimelidine, 20 mg/kg/24 hr, SC, for 14 days increased the duration of vaginal cycles in 3 month-old Long Evans hooded rats. It induced persistent vaginal estrus in 12 of 16 ten-month-old animals, and blocked reinitiation of vaginal cycles by L-dopa in 10 of 10 twenty-month-old rats. A single injection of zimelidine at 1400 hr did not alter the vaginal smear pattern of young or middle-aged cycling females or old constant estrus females. Also, a single dose of zimelidine at 1400 hr on the day of vaginal proestrus had no effect on serum LH values in young females. The serotonergic neurotoxin 5,7-dihydroxytryptamine, 4 μg, injected into the ventral and dorsal raphe areas (after desipramine, 25 mg/kg IP) reinitiated vaginal cycling in 8 of 13 twenty-month-old rats. These results suggest that (1) age-dependent changes in serotonin metabolism may contribute to the age-dependent changes in luteinizing hormone secretion which eventually lead to the cessation of ovarian function in the rat and (2) that alterations in serotonin function are an important component of the mechanism by which treatments with catecholamine precursors reinstate ovarian function in the old female rat.

Aging 5,7-Dihydroxytryptamine Serotonin Zimelidine Catecholamines Ovarian function

AGE-DEPENDENT changes in reproductive function of the female rat are typically noted beginning 10–12 months of age. At this time, the regular 4–5 day estrous cycle, present in the young female, is disrupted and the majority of animals become constant estrus (CE) [24]. The cessation of ovarian cycles in the middle-aged female is preceded by marked alterations in the pattern of LH secretion on the day of vaginal proestrus, which include a delay in the onset of the surge and lower peak values of this hormone [9].

The loss of ovarian cycles in the rat does not appear to occur as a function of changes intrinsic to the ovary. Transplantation of ovaries from old CE females into young, ovariectomized females results in regular cycling, whereas the reciprocal transplant of young ovaries into old, ovariectomized females will result in a resumption of the CE condition. In contrast, ovulation and/or vaginal cycles can be reinitiated in old CE females by systemic treatment with catecholamine (CA) precursors such as L-tyrosine or L-dopa [23], monoamine oxidase inhibitors [26], and dopamine (DA) receptor agonist [3,10]. These observations suggest that the disruption of regular ovarian function occurs as a consequence of age-related alterations in CNS neurotransmitter regulation of the anterior pituitary gland. This possibility is further supported by the finding that electrical stimulation of the medial preoptic area (MPOA), a CNS region involved in the cyclic release of gonadotropins, will induce ovulation in old CE females [4]. Similarly, placement of L-dopa, but not L-tyrosine, into the MPOA will reinstate regular cycling and ovulation in the old female rat [8].

The mechanism(s) involved in the effect of CA precursor treatment on ovarian cycling in the old rat remains to be determined. Systemic treatment with L-tyrosine or L-dopa [15,35] or direct placement of L-dopa into the brain [25] may lead to significant increases in CNS DA and NE metabolism. Studies of the young rat have shown that NE, particularly in the MPOA-anterior hypothalamus, facilitates luteinizing-hormone releasing hormone release, LH secretion, and ovulation [29]. The concentration of NE and DA in the hypothalamus of the old female is significantly lower than the hypothalamic concentration of these CAs in the young female [32]. Furthermore, compared to NE and DA turnover rates in young animals, CA turnover rates in the hypothalamus of middle-aged female rats are reduced in a number of hypothalamic regions when measured on the day of vaginal proestrus [7,34]. These observations suggest that the ability of the CA precursors to reinitiate ovarian cycles in the aged female is mediated through the effect of these agents on CNS-CA metabolism.

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Serotonin is also involved in the regulation of gonadotropin secretion. In the young female, enhanced CNS 5-HT metabolism on the afternoon of vaginal proestrous will inhibit LH release and block ovulation [2,22], whereas at other times during the cycle depletion of 5-HT will disrupt LH secretion and ovulation [13,18]. It is curious that those pharmacological treatments effective in reinitiating ovarian function in the aged female are also known to influence serotonin metabolism. For example, both systemically [11] and centrally [25] administered L-dopa reduces CNS 5-HT neurotransmission. Systemically administered DA receptor stimulants, such as bromocriptine, also lower CNS 5-HT metabolism [20]. Likewise, systemically administered L-tyrosine, which reinstates ovarian function in old rats, also lowers 5-HT metabolism through its effect on tryptophan transport into the CNS [12]. However, centrally administered L-tyrosine, which does not reinstate ovarian cycles in the aged female, does not reduce CNS 5-HT synthesis because it does not reduce tryptophan uptake into the CNS [36]. Finally, the dopamine-beta-hydroxylase blocker, fusaric acid, which reduces norepinephrine synthesis and the serotonin depleting effect of systemically administered L-dopa [19], blocks L-dopa induced vaginal cycling in old females as well [23]. These observations suggest that the ability of the CA precursor treatments to lower CNS 5-HT metabolism may be a critical component of the mechanism involved in the reinitiation of ovarian function in the old rat and that an age-dependent increase in sub-cortical 5-HT turnover [16,31] may contribute to the age-dependent disruption of ovarian function.

In the present study we investigated the effect of selectively stimulating or reducing CNS 5-HT metabolism on ovarian function in the female rat. First, we investigated whether or not enhancing CNS 5-HT activity would disturb ovarian function in the young female and block the ability of L-dopa in restoring ovarian cycles in the old female. Second, we tested whether or not selectively depleting CNS 5-HT activity in the old female would reinstate ovarian cycles.

METHOD

Animals

Cesarean derived Long-Evans female rats were obtained from the continuous colony housed in the Center for the Study of Aging and Human Development at Duke University Medical Center. Throughout the experimental period, the animal room was maintained on a 14 hr light:10 hr dark schedule (lights off at 1900 hr) and at 22±2°C. Water and Purina Rodent Lab chow 5001 (in pellet or powdered form) was provided ad lib.

Daily vaginal smears were obtained from all animals for three weeks prior to, during and, in some groups, up to 35 days after treatment was terminated. The smears were classified according to the criteria described previously [23]. Following the initial observation period only young (4 month old) and middle-aged (10.5 month old) female rats having regular 4-day vaginal cycles (i.e., two days of diestrus, 1 day proestrus and 1 day estrus) and old (20 months old) female rats showing constant vaginal estrus (CE) were included in the treatment groups.

Experiment 1

To evaluate the effect of enhancing CNS 5-HT stimulation on ovarian function, female rats were subjected to acute or chronic treatment with zimelidine, a relatively specific 5-HT reuptake inhibitor [27].

Acute zimelidine treatment. Groups of six young, middle-aged proestrous and old CE females were given a single subcutaneous injection of zimelidine at 1400 hr (20 mg/kg, dissolved in distilled water). Vaginal smears were monitored for a subsequent two week period. To determine the possible effect of the single treatment with zimelidine on the LH surge in young females, four serial blood samples were obtained at two-hour intervals from a second group of zimelidine-treated animals using a tail vein puncture technique described previously [9]. After sampling, the blood was centrifuged, the serum removed and stored frozen for subsequent LH determinations. Reference LH (rat LH-RP-1), purified LH for iodination (rat LH I-4), and LH I-4 antisera were obtained from the NIADDK and the National Pituitary Agency and were prepared by Dr. A. Parlow. LH was iodinated using the chloramine-T method [7]. RIAs were conducted according to NIADDK instructions. Intra- and interassay variabilities were 7% and 10% respectively.

Chronic zimelidine treatment. Daily, subcutaneous injections of zimelidine (20 mg/kg, dissolved in distilled water) were administered to groups of young, middle-aged and old female rats for 14 days. All injections were given at 1400 hr. Treatment was initiated in all young and middle-aged animals on the day of vaginal proestrus. The dose of zimelidine (20 mg/kg) used in these studies has been shown previously to enhance CNS 5-HT receptor stimulation while having relatively minor effects on CNS CA function [28]. There were 16 animals in each age × zimelidine-treatment group. Ten, age-matched control animals received daily injections of an equal volume of distilled water for a period of 14 days. The vaginal smears of all animals were also observed daily during the treatment period. On day 14, half the animals in each age group (N=8) were sacrificed by decapitation six hours after the last injection (i.e., 2000 hr, Day 14) in order to determine whether or not zimelidine levels in the serum were different in the three age groups. The ovaries of these females were processed and examined histologically. Trunk blood was collected in clean polypropylene tubes and centrifuged. The sera were stored at −60°C for subsequent zimelidine assay with a previously published liquid chromatographic procedure [33]. Zimelidine was extracted in an alkaline pH into diethylether-hexane which was evaporated, redissolved into the mobile phase, injected into a silica column and quantified by UV-detection. Daily vaginal smears were continued in the remaining 8 animals in each age group for 14 days after withdrawal of zimelidine.

During the treatment period, serum LH concentrations were determined in control and zimelidine-treated young animals on the day of proestrus by using the serial bleeding technique and LH RIA described above.

Experiment 2

The purpose of this experiment was to determine whether zimelidine injections would block the ability of L-dopa to reinitiate vaginal cycles in the old CE female. Twenty young, regularly cycling and twenty old, CE rats were placed on a plain powdered food diet for 14 days as described previously [5,6]. The vaginal smears of all animals were followed for this period. On Day 15, the powdered food of all animals was supplemented with Sinemet (Merck, Sharpe and Dohme) at a concentration 5 mg Sinemet per gram food. Sinemet is a combination of L-dopa and a peripheral dopa decarboxylase

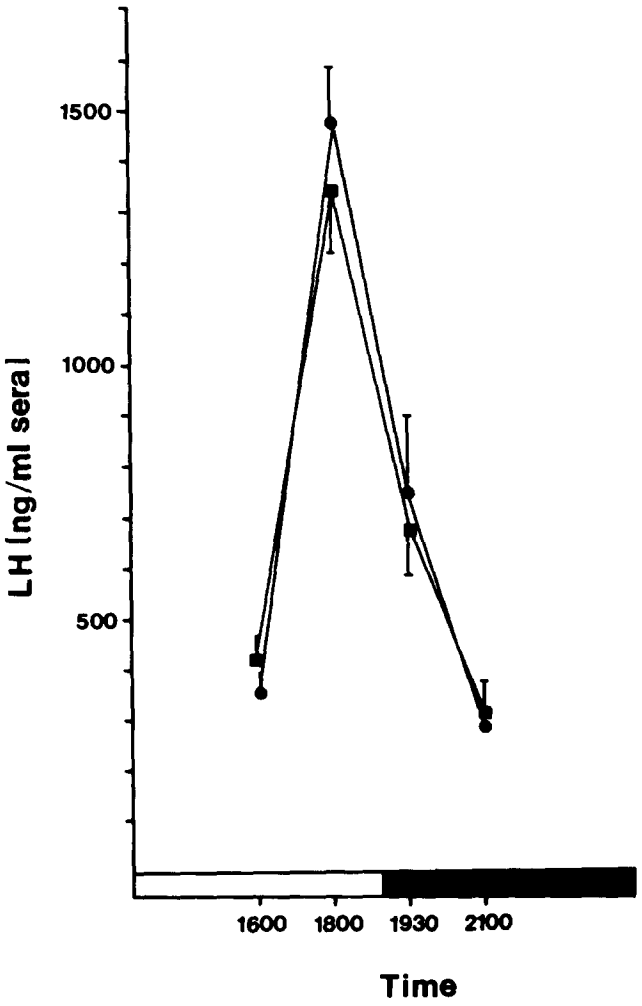

FIG. 1. Mean (±SEM) LH concentrations observed in young proestrous females receiving a single injection of zimelidine (circles) or vehicle (squares) at 1400 hr.

inhibitor, carbidopa (10:1). Half the animals in each age group fed the Sinemet-supplemented diet were also injected with zimelidine as described in Experiment 1. The zimelidine injections were initiated on the same day the Sinemet diet was introduced and both treatments continued for 14 days. The vaginal smears of these animals were observed during the treatment period and for a two-week period after treatment was stopped.

Experiment 3

In order to evaluate the effect of decreasing CNS 5-HT neuronal activity on ovarian function, young cycling and old CE females were treated with the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT), a compound that causes selective degeneration of central serotonin containing neurons [1]. All animals were pretreated with desmethylimipramine (DMI) (25 mg/kg, IP) 40–60 minutes prior to 5,7-DHT injections. By blocking the uptake of 5,7-DHT into noradrenergic neurons, DMI prevents destruction of that system and enhances the specificity of 5,7-DHT in lesioning serotonergic cells. Six young and 13 old females were anesthetized with Nembutal (50 mg/kg, IP) and 5,7-DHT, dissolved in 0.05% ascorbic acid, was injected into the ventral and dorsal raphe (4 $\mu g/\mu l$ vehicle into each area) by standard stereotaxic procedures. Six control animals in each age group received DMI and injections of only ascorbic acid into the raphe regions. The vaginal smear of each rat was followed for 35 days after surgery. On Day 35 all rats were sacrificed. The ovaries were removed and examined histologically.

Statistics

The effect of zimelidine or 5,7-DHT on the vaginal smear pattern was tested statistically using the Fisher exact probability test [30]. For young and middle-aged animals, this was accomplished by classifying the animals within the treatment groups as cycling (i.e., regular 4–5 day estrous cycles) or noncycling (i.e., prolonged diestrus, persistent and constant estrus). Drug-treated groups were then compared with the appropriate age-matched controls. The null hypothesis was that young animals treated with zimelidine or 5,7-DHT would have no effect on the vaginal smear pattern. Similar comparisons were made for old CE animals. That is, for zimelidine- or 5,7-DHT-treated animals, the null hypothesis was that these drugs would not alter the CE condition. Maximum LH levels in the young control and zimelidine, treated rats, and serum zimelidine levels in the young, middle-aged and old rats were compared using the Student's *t*-test for independent samples (two-tailed probability). Comparison between the time peak LH values observed in young control- and zimelidine-treated females was made using the Fisher exact probability test.

RESULTS

Experiment 1

Acute zimelidine treatment. A single injection of zimelidine (20 mg/kg) at 1400 hr did not affect the amplitude or timing of the proestrous LH surge in young females (Fig. 1). A single injection of zimelidine also did not alter the vaginal smear pattern in the young and middle-aged, regularly cycling females or the old CE females during the two-week postinjection period.

Chronic zimelidine treatment. Regular 4-day vaginal cycles continued in all the control-treated, young females. In contrast, regular vaginal cycles were disrupted in a significant number of the young animals receiving chronic zimelidine treatment ($p<0.0001$; see Table 1). This was evidenced by prolonged diestrous (range 2–9 days, mean=3.7±0.4 days) and estrous (range 1–5 days, mean=1.94±0.4 days) periods in the zimelidine-treated rats compared to two day diestrous and 1 day estrous periods in control animals. Serial blood samples were obtained on the day of vaginal proestrus from nine zimelidine-treated and 6 control females during the treatment period. An LH surge was noted in all six control animals but in only 6 of the nine zimelidine-treated rats. The LH values observed during the peak were not different between the two groups, however, the time at which the peak value was observed was different between the two groups ($p<0.001$) with the zimelidine-treated animals reaching peak LH levels later than the control animals, indicating that the surge was delayed in these females (Fig. 2). Regular 4-day vaginal cycles returned within one week after zimelidine withdrawal in all 8 females observed during the post-treatment period.

TABLE 1

EFFECT OF ZIMELIDINE ON VAGINAL CYTOLOGY OF LONG EVANS RATS

			Vaginal smear pattern during two week treatment period*			
Age	Group	N	Regular cycles	Irregular cycles	Persistent or constant estrus§	Predominantly leukocytic
4 months	Control†	10	10	0	0	0
	zimel. acute	8	8	0	0	0
	zimel. chronic	16	3	9	3	1
	Sinemet‡	10	9	0	1	0
	Sin + zimel.	12	6	6	0	0
10 months	Control	11	9	0	1	1
	zimel. acute	8	7	1	0	0
	zimel. chronic	16	0	4	12	0
19 months	Control	10	0	0	10	0
	zimel. acute	8	0	0	0	8
	zimel. chronic	16	0	0	0	16
	Sinemet	10	4¶	2	4	0
	Sin + zimel.	10	0	0	0	10

*Numbers indicate animals showing each smear pattern after treatment.

†Control rats received 0.5 ml distilled water.

‡Sinemet was administered in the diet (500 mg Sinemet/100 g powdered food).

§Persistent estrus=2–7 days of cornification, constant E=cornification throughout observation period.

¶In two rats cycles were 5 days in length (i.e., 3 days of diestrus).

The number of middle-aged animals showing regular vaginal cycles during the treatment period was significantly greater in the control group than in the zimelidine-treated group ($p<0.0001$). Zimelidine disrupted regular cycling in all 16 middle-aged females producing persistent vaginal estrus (range 5–12 days, mean 7.5 ± 1.7 days) in 12 females while 4 of the 16 middle-aged animals treated displayed irregular vaginal cycles (i.e., 4–6 days of diestrus, or 2–3 days of cornification). Regular cycling resumed within one week of treatment withdrawal in 6 of the 8 females followed.

Zimelidine treatment of old CE females led to a mucification of the vaginal smear in all females within three days after treatment was initiated. Inspection of the ovaries of the females sacrificed on Day 14 revealed tissue devoid of corpora lutea with and numerous atretic follicles. The vaginal smears of the remaining old females remained mucified during the treatment period and throughout the two-week post-treatment period in six of the 8 females observed. Constant vaginal cornification returned in the remaining two females within 7 days of treatment withdrawal.

Determination of serum zimelidine and norzimelidine concentration revealed that the concentration of both these substances was significantly lower in young females (zimelidine=180 ± 136 ng/ml; norzimelidine=5194 ± 541 ng/ml) than the middle-aged ($p<0.01$) or old ($p<0.001$) females. The levels of zimelidine and norzimelidine in the two older groups were not different (combined mean for zimelidine=663 ± 223 ng/ml; combined mean for norzimelidine=6275 ± 544 ng/ml).

Experiment 2

Regular 4-day cycles continued in the young control females when their diet was changed from pellet to powdered food. Similarly, changing the diet of old CE females from pellet to powdered food had no effect on the vaginal cytology. The effect of the Sinemet-supplemented diet on the reproductive status of young, cycling and old CE females is also shown in Table 1. Nine of the ten young females fed the powdered food diet (supplemented with 5 mg Sinemet/gram food) continued to cycle regularly. One young female became CE and remained so throughout the treatment and post-treatment period. The Sinemet-supplemented diet also induced changes in the vaginal smear of six of the ten old females (Sinemet vs. controls, $p<0.04$). In four old females 4 or 5 day vaginal cycles were observed during the treatment period, two old females showed one vaginal cycle each and four remained constant estrus throughout the treatment and post-treatment periods. All old females resumed the CE condition within 8 days after the Sinemet-supplemented diet was withdrawn.

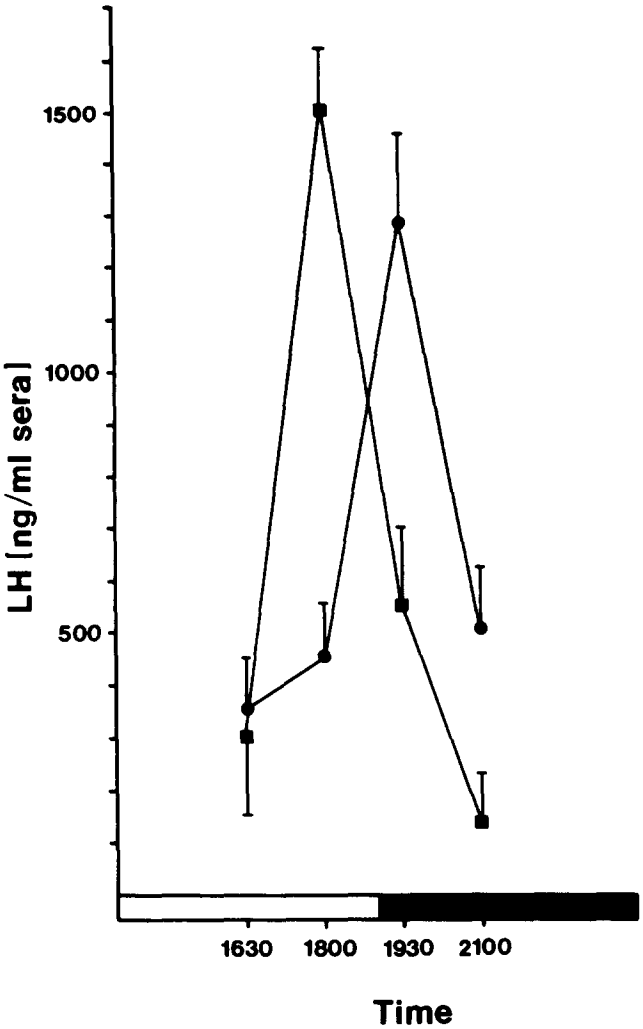

FIG. 2. Mean (±SEM) LH concentrations observed in young proestrous females after continued daily treatment with zimelidine (circles) or vehicle (squares).

Feeding young females the Sinemet-supplemented diet appeared to partially block the disruptive effect of zimelidine treatment. That is, during the treatment period, the number of females showing regular vaginal cycles (i.e., 50%) in the Sinemet plus zimelidine group was significantly greater ($p<0.007$) than the number of females showing regular cycles in the zimelidine only group (19%). However, the Sinemet-supplemented diet did not completely inhibit the disruptive effect of zimelidine treatment on vaginal cycling in the young females. The six females in which cycling was disrupted during this treatment combination revealed prolonged periods of diestrus (range 2–11 days, mean=3.7 days) and estrus (range 1–4 days, mean=1.5 days). Thus, compared to the young control or young rats fed Sinemet only, significantly fewer rats ($p<0.05$) receiving the combined Sinemet- and zimelidine-treatment showed regular vaginal cycles during the treatment period.

The effect of combined Sinemet and zimelidine treatment on the vaginal cytology of the old CE females was the same as that observed in the old CE females receiving zimelidine alone. That is, within three days of being subjected to the combined treatment of Sinemet and zimelidine, the vaginal smear of all ten old CE females became heavily mucified and remained so throughout the duration of the treatment and post-treatment period. These results also demonstrate that zimelidine treatment of old CE females placed on the Sinemet-supplemented diet blocked the ability of this diet to reinstate vaginal cycles (i.e., comparing Sinemet-treated with Sinemet- plus zimelidine-treated CE females, $p<0.002$).

TABLE 2

EFFECT OF 5,7-DHT ON VAGINAL CYTOLOGY OF LONG EVANS RATS

Rats/ Treatment	N	Proportion of females showing regular 4 or 5 day vaginal cycles* (0–14 days post-op)	(15–35 days post-op)
Old CE 5,7-DHT	13	3/13	8/13†
Old CE sham-op	6	0/6	0/6
Young 5,7-DHT	6	6/6	6/6
Young sham-op	6	6/6	6/6

*Regular vaginal cycles defined as 2–3 complete cycles within a 14 day period.

†$p<0.01$ comparing the number of females cycling before surgery with the number cycling 15–35 days after surgery.

Experiment 3

The effect of treatment with the neurotoxin 5,7-DHT on vaginal cycling in young and old animals is shown in Table 2. During the first two postoperative weeks, regular (i.e., 4–6 day vaginal cycles) were observed in 23% (3/13) of the old 5,7-DHT-treated females; between the third and fifth post-operative weeks, 62% of the old 5,7-DHT-treated females showed vaginal cycling. None of the old sham-operated showed vaginal cycling during the postoperative period, however, the surgery did cause prolonged diestrous periods in three females. Treatment with 5,7-DHT, or ascorbic acid alone, did not disrupt regular cycling in the young females. Histological examination of the old females' ovaries revealed that corpora lutea were present in the ovaries of the old females showing regular vaginal cycles indicating that some of these cycles were ovulatory. The ovaries of the nonresponding females contained numerous, well-developed follicles but no corpora lutea.

DISCUSSION

The results of the above experiments suggest a role for 5-HT in both the initial disruption of ovarian cycling as the female rat ages and the reinitiation of ovarian function in the old female once cycling has stopped. The 5-HT reuptake blocker zimelidine altered ovarian-vaginal cycles in the majority of animals in all three age groups. However, the pattern of change differed depending on the animal's age at the time of treatment. Zimelidine treatment of young animals produced irregular vaginal cycles and a delay in the onset of the proestrous LH surge, changes typically seen in middle-aged females. Zimelidine treatment to the middle-aged

females produced persistent or constant vaginal cornification, a condition commonly seen in older females in whom regular cycling had already ceased. Finally, zimelidine treatment in the old CE female produced leukocytic smears, a pattern usually observed in very old females. These changes indicate that treatment with agents that enhance CNS 5-HT neural activity facilitates reproductive aging in the female rat.

The observation that zimelidine treatment to old CE females fed a Sinemet-supplemented diet blocked the ability of this diet to reinstate ovarian cycles indicates that a reduction in CNS 5-HT activity may be a critical component of the mechanism by which treatment with CA precursors act to reinstate ovarian cycles in old females. Zimelidine, and particularly norzimelidine, are approximately ten times more potent in inhibiting 5-HT reuptake than norepinephrine reuptake [27]. It is unlikely that the effect of zimelidine on NE function contributed to the present observations. First, the dose of zimelidine used in the above experiments is reported to cause a minor (i.e., less than 20%) reduction in norepinephrine beta receptor binding in the rat brain [28]. Second, a single injection of zimelidine had no effect on the proestrous LH surge in young adult females when administered during the critical period, the time at which acute treatment with CA blocking agents has been shown to inhibit the LH surge [21]. However, the concentrations of zimelidine and norzimelidine in the young rat were below those observed in the sera of middle-aged and old rats. Thus, the difference between a change in estrous cyclicity in the young and middle-aged female and the changes in the vaginal smear pattern observed in the old female may have been due, in part, to a differential stimulation of 5-HT activity and indirectly to a greater effect on catecholamine activity in the three age groups. At this point, this possibility can not be discounted.

Although NE is critical to LH release [29] and NE content in the intact female rat's brain is decreased with age [32], we found previously that treatment with alpha (phenoxybenzamine) and beta (L-propranolol) noradrenergic receptor blockers did not alter the ability of L-dopa to reinitiate vaginal cycles in old CE females [23]. Also, simultaneous treatment with L-dopa and the dopamine beta hydroxylase inhibitor, fusaric acid, blocked the ability of L-dopa to reinitiate ovarian cycles [23]. Fusaric acid has also been shown to block the 5-HT depleting effect of L-dopa [19]. Finally, zimelidine treatment did block the ability of the Sinemet-supplemented diet to restore reproductive cycles in the old female. Theoretically, this effect may have been mediated either through a direct and continuous stimulation of postsynaptic 5-HT receptors due to a reuptake inhibition of the amine or through the ability of zimelidine to block the uptake of L-dopa into serotonin containing neurons, thus blocking the serotonin depleting effect of L-dopa. In summary, these observations would indicate that the effect of zimelidine on reproductive function observed in the present studies is mediated primarily through the effect of this drug on serotonin metabolism.

The fact that chronic zimelidine treatment caused a delay in the onset of the LH surge in the young female rats again suggests that this drug produces changes within the central nervous system that are functionally similar to the changes that occur during normal aging. We found previously that there is a delay in the onset of the proestrous LH surge in the middle-aged female [6]. It is possible that these shifts in the timing of the LH surge represent age-dependent changes in the diurnal pattern of serotonin and catecholamine rhythms in the brain and subsequently altered release of luteinizing hormone releasing hormone. We had previously hypothesized that such shifts occur because a functional imbalance develops between the serotonergic and catecholaminergic control of LH release [5].

Further support for the argument that the effect of zimelidine is mediated primarily through this drug's effect on serotonin neurons is suggested by the anecdotal observation that most of the old rats used in these studies displayed behavioral symptoms of the serotonin syndrome [14] towards the end of the two-week treatment period. Such behavioral effects were not observed in the middle-aged and young females treated with zimelidine. This observation would also suggest that the endogenous level of serotonin metabolism is increased in the older female relative to the young, however, the possibility that the higher zimelidine and norzimelidine levels observed in the older animals were responsible for the different behavioral sensitivities of the groups to zimelidine can not be excluded.

Additional support for the hypothesis that part of the mechanism involved in the ability of CA precursors to reinitiate ovarian cycling in old females involves a reduction of serotonin is provided by the studies using the neurotoxin 5,7-DHT. Selectively decreasing CNS 5-HT in old CE females resulted in a reinitiation of ovarian cycles as indicated by vaginal cycling and the presence of corpora lutea in the ovaries of old, previously CE, 5,7-DHT treated females. Thus, reducing CNS 5-HT neural activity alone is sufficient to reinitiate ovarian cycling. Again, these observations indicate that with age a functional imbalance develops between the CA and 5-HT control of LH release such that initially, there is a shift in the timing of the LH surge on the day of vaginal proestrus. As this imbalance increases, with age, normal LH release is completely disrupted and a pattern of CE ensues. The development of this imbalance can be delayed by treatments that simultaneously increase CNS CA and reduce 5-HT metabolism [5,6] or by treatments designed to selectively increase CNS CA metabolism or selectively decrease CNS 5-HT metabolism. Obviously, further studies involving direct measurements of these CNS monoamines in the aging female's brain are needed to determine the precise nature of the functional changes that occur in these systems.

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