Adrenergic Activity in Hypothalamus and Ovulation[1] (35110)

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(Introduced by S. M. McCann)

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Norepinephrine (NE) levels in the anterior and middle hypothalamus show obvious changes during the estrous cycle in rats (1). These modifications were suppressed by ovariectomy (2) or by neonatal injection of testosterone (3). It was recently observed (4) that the intensity of the catecholamine (CA) fluorescence in the median eminence increases from the first day of diestrus to the day of estrus suggesting activity changes of the tuberoinfundibular neurons. Characteristic changes in these neurons were observed in the guinea pig hypothalamus by using the histochemical fluorescence method (5). In addition, considerable evidence indicates that adrenergic blocking drugs can suppress ovulation both in immature rats treated with PMS gonadotropin (6, 7) and in adult rats treated with the blockers at the critical period (8). All these findings support the hypothesis that catecholamines may be linked with other brain mechanisms which control cyclic secretion of gonadotropins by the pituitary.

The purpose of this work was to study the turnover of endogenous norepinephrine and its synthesis in the hypothalamus in rats just prior to (proestrus) and subsequent to ovulation (estrus).

Materials and Methods. Adult female Holtzman rats weighing 170–200 g were used. The animals were housed in a room with controlled lighting (14 hr light and 10 hr darkness) and temperature (22–25°). They were fed Forramez laboratory rat chow and water *ad libitum*. Vaginal smears were taken daily. Animals which displayed at least two normal 4-day vaginal cycles were used in the experiments. At autopsy, uteri were observed for ballooning.

Norepinephrine in each hypothalamus was fluorimetrically assayed (9) after extraction in 0.4 *N* perchloric acid. To evaluate the turnover of hypothalamic norepinephrine, α-methyl-*p*-tyrosine (MPT) methylester hydrochloride[4] which blocks catecholamine biosynthesis, was injected ip dissolved in saline at doses of 300 mg/kg. Rats were sacrificed 4 hr after injection. Untreated control rats were simultaneously sacrificed to calculate the steady state level of NE and the rate of depletion in the MPT-treated rats.

The synthesis of NE was estimated as follows: Tritiated tyrosine (^{3}H-Ty) was injected iv into rats anesthetized with ether at doses of 100 μCi (sp act, 49 Ci/mmole; The Radiochemical Centre, Amersham, England). Three hr later, rats were sacrificed and the hypothalamus was dissected out as previously described (10). Extracts were prepared in 0.4 *N* perchloric acid from five pooled hypothalami from proestrus and estrous rats. A complementary experiment was done with rats sacrificed on day 2 of diestrus. All experiments were performed between noon and 4:00 p.m.

Results. Turnover of endogenous norepinephrine. Hypothalamic norepinephrine concentration in proestrous rats was higher than

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TABLE I. Effect of Blockade of Synthesis of Norepinephrine by α-Methyl-*p*-tyrosine on the Norepinephrine Content in Rat Hypothalamus at Two Stages of the Estrous Cycle.

Phase of the cycle	Norepinephrine conc (μg/g)		Mean decrease of NE 4 hr after MPT	
	Control	After MPT	(μg/g)	(%)
Proestrus	3.48 ± 0.09 (7)[a,b]	1.31 ± 0.12 (7)	2.17	62.4
Estrus	2.13 ± 0.04 (7)	1.52 ± 0.06 (7)	0.61	28.6

[a] Mean ± SEM (7 rats/group).
[b] $p < 0.01$ vs estrus.

in the estrous rats (Table I). When MPT was administered at noon and the animals killed 4 hr later, a clear decrease in NE levels was observed. Proestrous rats showed a twofold greater decline of the NE concentration than estrous rats. As the initial levels of hypothalamic NE differed significantly ($p < 0.01$) between these two groups, whereas the values at the fourth hour after MPT injection were similar, it could be inferred that proestrous rats show an accelerated NE turnover as compared with rats studied during the estrous phase.

Biosynthesis of norepinephrine in hypothalamus. After injection of tracer amounts of tritiated tyrosine a higher rate of NE synthesis was produced in the hypothalamus during proestrus compared to that observed at estrus (Table II). An unexpected finding was the high value obtained during diestrus day 2 in which the synthesis of NE was similar to that obtained at proestrus.

Discussion. The present observations reporting a high content of NE in hypothalamus during proestrus are in agreement with our previous findings (1). In addition we have reported that the increase of NE levels between diestrus day 2 and proestrus is localized to the anterior and middle hypothalamus.

TABLE II. Synthesis of Norepinephrine (^{3}H-NE) from ^{3}H-Tyrosine in Hypothalamus at Different Stages of the Estrous Cycle.

	^{3}H-NE (mμCi/g)	Wt of tissue sample (mg)
Proestrus	2.7 (1)[a]	76.0
	2.9 (3)	65.0
Estrus	1.8 (1)	73.9
	1.9 (2)	77.5
Diestrus Day 2	3.4 (3)	73.7
	3.6 (4)	75.5

[a] Experiment no.: where the numbers are the same, the groups were run in the same experiment.

By following the decline of NE levels in hypothalamus after blockade of catecholamine synthesis by *a*-methyl-*p*-tyrosine it is possible to estimate their rate of turnover under different conditions. It is then assumed that a direct relationship exists between the metabolism of the catecholamine and its rate of synthesis. According to this concept the degree of amine depletion is highly dependent on the degree of sympathetic activity (11).

It has been previously reported that hypothalamic norepinephrine shows an increased rate of turnover in ovariectomized rats as determined by the fall in NE after α-methyl-*p*-tyrosine (6, 12). Recently, direct measurements of NE formation in hypothalamus from its natural precursor (10) or following the decline of specific activity of ^{3}H-NE injected into the lateral ventricle of brain (13, 14) give evidence supporting the view that castration accelerates the rate of NE synthesis. The present paper shows that before ovulation, *i.e.*, at proestrus, both a marked increase in the rate of turnover and synthesis of NE (^{3}H-NE) from tyrosine (^{3}H-Ty) takes place in normal rats. It seems also that an increased adrenergic activity begins to operate before proestrus; the results obtained during diestrus day two (Table II) suggest this view.

Contradictory observations on the behavior of the catecholaminergic neurons in hypothalamus of rats during the estrous cycle have been recently reported. According to

Fuxe and Hokfelt (15) the rate of depletion of the fluorescence after CA synthesis blockade by α-methyl-*p*-tyrosine is higher in the tuberoinfundibular catecholaminergic neurons at diestrus than at proestrus and estrus, a conclusion opposite to that reached by Lichtensteiger (4). This author, by means of microfluorescence methodology states that a progressive increase in functional activity occurs from diestrus day 1 to estrus as indicated by the fluorescence intensity. However, his results obtained at proestrus and estrus do not disclose any difference in fluorescence between these two phases. As was reported previously, hypothalamic NE concentration (1) and monoamine oxidase activity (16) are higher in proestrus than in estrus.

It is probable that our results are mainly related to changes in the afferent NE termials in the hypothalamus (15) and to changes in adrenergic neurons placed in the basal hypothalamus, but it may well be that other neurons, *i.e.,* tuberoinfundibular cells, may also be involved. Our previous results (1) showed that NE concentration increases in the middle hypothalamus before ovulation. Moreover, according to some authors (17, 18) NE contributes significantly to the total CA contained in that region.

Until now it has not been possible to draw definite conclusions about the role of brain catecholamines in the control of gonadal function; however, an active (facilitatory) adrenergic tone seems to be related to the secretion of pituitary gonadotropins (LH and FSH). Studies supporting this hypothesis have been recently published (19–21).

Summary. Adrenergic activity of the hypothalamus prior to and subsequent to ovulation was evaluated in rats. Turnover of hypothalamic norepinephrine was measured by following the decline of endogenous NE levels after synthesis blockade with α-methyl-*p*-tyrosine methylester. Proestrous rats showed both a greater concentration and depletion of NE than estrous rats. Coincidentally, in proestrous rats an increased synthesis of hypothalamic NE as measured from the conversion of ^{3}H-tyrosine to ^{3}H-NE was observed. These results suggest that an increased NE activity takes place in the rat hypothalamus before ovulation.

1. Stefano, F. J. E., and Donoso, A. O., Endocrinology **81**, 1405 (1967).

2. Donoso, A. O., and Stefano, F. J. E., Experientia **23**, 665 (1967).

3. Donoso, A. L., and Cukier, J., Nature (London) **218**, 969 (1968).

4. Lichtensteiger, W., J. Pharmacol. **165**, 204 (1969).

5. Leonardelli J., Ann. Endocrinol. **30**, 783 (1969).

6. Coppola, J. A., J. Reprod. Fert. Suppl. **4**, 35 (1968).

7. Kordon, C., and Glowinski, J., Endocrinology **85**, 924 (1969).

8. Meyerson, B. J., and Sawyer, C. H., Endocrinology **83**, 170 (1968).

9. Laverty, R., and Taylor, K. M., Anal. Biochem. **22**, 269 (1968).

10. Donoso, A. O., De Gutierrez Moyano, M., and Santolaya, R. C., Neuroendocrinology **4**, 12 (1969).

11. Brodie, B. B., Costa, E., Dlabac, A., Neff, N. H., and Smookler, H. H., J. Pharmacol. **154**, 493 (1966).

12. Coppola, J. A., Neuroendocrinology **5**, 75 (1969).

13. Anton-Tay, F., Pelham, R. W., and Wurtman, R. J., Endocrinology **84**, 1489 (1969).

14. Wurtman, R. J., Anton-Tay, F., and Anton, S., Life Sci. **8**, 1015 (1969).

15. Fuxe, K., and Hokfelt, T., *in* "Frontiers in Neuroendocrinology" (W. F. Ganong and L. Martini, eds.), p. 47. Oxford Univ. Press, London/New York (1969).

16. Kobayashi, T., Kobayashi, T., Kato, J., and Minaguchi, H., Endocrinol. Jap. **11**, 283 (1964).

17. Rinne, U. K., and Sonninen, V., Experientia **24**, 177 (1968).

18. Iwata, T., and Ishii, S., Neuroendocrinology **5**, 140 (1969).

19. Schneider, H. P. G., and McCann, S. M., Endocrinology **85**, 121 (1969).

20. Kamberi, I. A., Mical, R. S., and Porter, J. C., Science **166**, 388 (1969).

21. Schneider, H. P. G., and McCann, S. M., Endocrinology **86**, 1127 (1970).

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