Natural Variations in Brain Phenylethanolamine-N-Methyltransferase Activity During Different Phases of the Oestrous Cycle: Effect of Chronic Dexamethasone Treatment

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Abstract—The activity of phenylethanolamine-N-methyltransferase (PNMT) was studied in the hypothalamus, hypophysis, striatum and the rest of the brain during the four different phases of the oestrous cycle of the rat. The oestrous phase was marked by high level of enzyme activity in all the regions except the hypophysis. The hypophysis showed maximum increase in activity at the metoestrous phase of the oestrous cycle. Chronic treatment of rats with dexamethasone for 10 days led to decreased level of PNMT activity in the hypophysis, hypothalamus and the rest of brain. These decreases were important in the hypothalamus and the hypophysis which represented only 25 and 20% of the control activity after dexamethasone administration.

Phenylethanolamine-N-methyltransferase (PNMT) is responsible for the N-methylation of noradrenaline to adrenaline (1-3). It is mainly localized in the adrenal medulla where it is present in maximum activity (4, 5). Some traces of this enzyme were reported to be detectable in the heart and central nervous system (6). The presence of PNMT in the brain regions was further supported by the demonstration of adrenaline stores in the central nervous system by several groups of investigators (7-9). The recent progress in the analytical method for PNMT has enabled the determination of slight traces of its activity in a wide range of brain nuclei (10-12). This enzyme of adrenaline synthesis in the periphery is highly affected by any change in the neuroendocrine status of the organism (13, 14). Now it is well established that a number of steroid and peptide hormones induce the protein synthesis of PNMT in the adrenal medulla (15). However, such a regulation of PNMT in the brain regions by modified endocrine secretions still remains to be clearly understood. The present investigation was designed to observe such variations in PNMT of brain regions under very simple physiological conditions such as the oestrous cycle which is accompanied by significant changes of the content of different steroid hormones (16-18). Natural modifications in enzyme activity in the hypophysis, hypothalamus, striatum and the rest of the brain were observed during the metoestrous, dioestrous, prooestrous and oestrous phases of the cycle. A group of female rats were administered chronically with dexamethasone for 10 days to see if this synthetic steroid has some influence on cerebral PNMT activity.

Materials and Methods

Albino female rats of the Sherman strain were utilized in all experiments. The weight of the animals ranged between 250 to 280 g. The rats were kept at a constant temperature of 21°C with exposure to natural day and night cycles. The females in different phases of the oestrous cycle were separated by microscopic examination of vaginal smears.
The rats for the oestrous phase were obtained mostly in the night. Only the animals with very distinct characterization of the specific phase of the oestrous cycle were selected for PNMT assays. At each defined phase, the rats were sacrificed by neck fracture, decapitated, and the brain was dissected on ice. Hypophysis, hypothalamus, striatum and the rest of brain were homogenized immediately in ice cold 0.9% KCl.

**Dexamethasone administration:** Dexamethasone as an injectable solution was supplied generously by Merck, Sharp and Dohme Laboratories, France. The rats were administered daily with 160 μg/100 g of body weight of dexamethasone subcutaneously for a duration of 10 days. After the termination of this chronic treatment, only females in the metoestrous phase were taken for the determination of PNMT activity. It was observed that most of the females receiving this dose of dexamethasone were in the metoestrous phase of the oestrous cycle. This particular dose was selected to have toxic effects of dexamethasone during pregnancy.

**Determination of PNMT:** The activity of PNMT was measured according to our microradioisotopic assay which employs methyl-\(^{14}\)C-S-adenosylmethionine as a methyl donor to normetanephrine used as a substrate (19, 20). The details of PNMT preparation from brain tissues have already been described elsewhere (10). The homogenization of hypophysis, hypothalamus, striatum and the rest of brain was made in ice-cold 0.9% KCl (50 mg/ml). The homogenate was centrifuged at 50,000 x g for 30 min, and the supernatant was employed as the enzyme preparation. The incubation mixture consisted of 0.2 ml of phosphate buffer (0.2 M, pH 7.9), 20 μl (0.1 μmole) of DL-normetanephrine, 50 μl (0.1 μCi) of methyl-\(^{14}\)C-S-adenosylmethionine (specific activity: 45 mCi/mMole) and 50 μl of enzyme preparation as described previously. The tubes were incubated for 1 hr at 37°C. The reaction was stopped by transferring the tubes to an ice bath for a few minutes and addition of 0.5 ml of borate buffer (1 M at pH 10). Three ml of toluene/isoamyl alcohol (3/2 ratio) were added to each tube and shaken vigorously on a mechanical shaker for 20 min. After centrifugation, 2 ml of the organic phase were extracted and transferred to a mini scintillation vial containing 3.5 ml of scintillation solution (10) and counted for 10 min. The method was highly reproducible, and the recovery was linear for different concentrations of the enzyme used for the standard curve. Blanks were prepared without DL-normetanephrine to omit nonspecific methyl transferases.

**Protein determination:** Tissue proteins were measured according to the method of Lowry et al. (21).

**Statistical method:** All results were subjected to statistical analysis according to Fisher's t-test. The mean values have been expressed with standard errors (S.E.M.).

**Results**

**Natural variations in PNMT during oestrous cycle:** Figure 1 illustrates the natural variations in PNMT activity of the entire hypothalamus that take place during the

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**Fig. 1.** Activity of the enzyme phenylethanolamine-N-methyltransferase (PNMT) in the hypothalamus of female Sherman rats during different phases of the oestrous cycle. The enzyme activity is expressed as % of the metoestrous phase (MST) which is taken as 100%. DST (dioestrous), PST (prooestrous), and OST (oestrous phase); n (number of rats used in each group). The following groups differ statistically: MST vs. PST: P<0.001, DST vs. PST: P<0.001, MST vs. OST: P<0.001.
four phases of the oestrous cycle. Prooestrous and oestrous phases of the cycle were accompanied by high rises of enzyme activity from the metoestrous and dioestrous values, and these changes were statistically significant.

Figure 2 shows PNMT activity in the striatum of rats during four phases of the oestrous cycle. Only the oestrous phase showed an increase in activity from the values of the three other phases.

Variations in hypophyseal PNMT activity are illustrated in Fig. 3. There was a marked and significant decrease in PNMT activity of this structure at the onset of the dioestrous phase of the oestrous cycle (~25%). Prooestrous and oestrous phases demonstrated further decreases in enzyme activity from the metoestrous value (about 40%). These variations were significant statistically.

Figure 4 provides changes in PNMT activity in the rest of the brain during various phases of the oestrous cycle. The enzyme activity did not vary significantly during the metoestrous, dioestrous and prooestrous phases. However, the oestrous phase showed a high rise in PNMT activity from the dioestrous and metoestrous values.

Chronic dexamethasone administration and PNMT activity: Figure 5 illustrates the effects of 10 days of chronic administration of dexamethasone (160 μg/100 g body weight, daily) upon PNMT activity of different brain regions. Marked decreases in enzyme activity took place as a consequence of this hormonal treatment. The maximal decrease was observed in the hypophysis which showed 75% lower activity. The decrease in hypothalamic PNMT was 70%, and the rest of the brain showed a 52% decline.
Fig. 5. Influence of dexamethasone treatment to female Sherman rats upon PNMT activity of the brain, hypothalamus and hypophysis determined during the metaoestrous phase of the oestrous cycle. The females received 160 mg/100 g of dexamethasone daily for 10 days subcutaneously. Statistically all the tissues showed decline in PNMT of the brain regions after treatment with this synthetic glucocorticoid. The higher dose was selected due to the fact that dexamethasone has toxic effects during pregnancy. Control vs. dexamethasone in rest of brain: P<0.001, in hypothalamus: P<0.001 and in hypophysis: P<0.001.

Discussion

Present findings suggest that the epinephrine forming enzyme in the brain regions can be greatly modified by any change in the endocrine status of the animals. PNMT is very important in determining the fate of vasoactive amines (22, 23), and the adrenal medulla which is the main source of PNMT activity requires a constant supply of corticoids for its induction (13, 14). Pharmacological or surgical suppression of the adrenal cortex decreases PNMT (14, 24–26). Glucocorticoids are the most potent hormones for PNMT induction (27, 28), but other steroids can also influence its activity (29–31). High doses of corticoids inhibit PNMT activity (13) by creating negative feedback. We employed a very high dose of dexamethasone to study pharmacological effects in the brain regions. The role of different phases of the oestrous cycle on brain PNMT appears to be associated with an overall shift of different steroid hormones taking place naturally during different phases of the oestrous cycle (16–18). The different response of PNMT activity in the hypothalamus and hypophysis during various phases of the oestrous cycle can be related to the different natures of steroid receptors present in these two central nuclei (24). Now it has been suggested that the nuclear receptors of the hypothalamus and hypophysis have different physiological response to progesterone treatment (24). A similar correlation between natural steroid variations and the activities of the enzymes monoamine oxidase and catechol-O-methyltransferase was also found during the oestrous cycle in the rat (32, 33). The latter authors showed that monoamine oxidase in the hypothalamus was very high at the prooestrous and dioestrous phases, whereas at oestrous and metaoestrous phases, the activity of this enzyme of catecholamine deamination reached its lowest level (32). The rise in monoamine oxidase activity at prooestrous and dioestrous phases was equally high in other brain nuclei such as the caudate nucleus and the septum (32). It was found that all such increases in monoamine oxidase activity were caused by a natural rise of the progesterone content in the ovaries and plasma (32) since its fall was associated with a decrease of enzyme activity during the oestrous phase.

Our past published observations support that progesterone augments whereas oestradiol inhibits monoamine oxidase activity (34) when given exogenously. This is why it is feasible to suggest that PNMT activity in the brain regions also depends to some extent on the endocrine changes that take place during the oestrous cycle. Such an observation can be supported by studies involving the measurements of brain noradrenaline and adrenaline during different phases of the oestrous cycle since the variation in the ratio of noradrenaline to adrenaline serves as a good index for N-methylation by the enzyme PNMT (35, 36).

The action of chronic administration of dexamethasone to rats in the oestrous cycle on central PNMT activity appears to be dependent on the status and function of the
hypothalamo-pituitary-adrenal function which has been shown to be impaired after therapy with corticosteroids or corticotrophin (37). These authors observed that beta methasone-treated rats showed adrenal atrophy and insensitivity to exogenous corticotrophin. In addition to this, similar treatment with the synthetic corticoid prevented the normal circadian rhythm and the stress-induced rise in plasma corticosterone concentration (37). The impairment of hypothalamo-pituitary-adrenal function resulting from chronic dexamethasone administration prevents the mobilization of endogenous corticotrophin which is required to maintain normal corticosterone levels in the plasma as well as in the central nervous system. It is well established that pharmacological doses of natural or synthetic glucocorticoids can act to provoke the atrophy of the adrenal cortex. When the circulating levels of corticoids are low, the activity of the enzyme PNMT goes down rapidly (2, 4). This is why we observe lower levels of brain PNMT in response to dexamethasone administration. Such an administration inhibits ACTH secretion which plays an important role in the induction of the enzyme PNMT (6).

From the present observations, it can be concluded that natural variations in steroid hormones during the four different phases of the oestrous cycle significantly affect central PNMT activity. Chronic dexamethasone administration is associated with significant decreases of PNMT activity in the brain regions, and this effect is most probably related to the impairment of the hypothalamo-pituitary-adrenal function caused by pharmacological doses of the steroid. This data gives further evidence that steroids play an important role for the maintenance of the enzyme PNMT, not only in the peripheral nervous system, but also in the central regions. Such modifications in N-methylation of noradrenaline to adrenaline could have important physiological consequences such as cerebral blood flow and glucose availability.

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