THE EFFECTS OF THE ESTROUS CYCLE AND AGING ON DOPA-DECARBOXYLASE ACTIVITY IN HYPOTHALAMIC NUCLEI

by

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INTRODUCTION

Many health problems are associated with aging including depression, Parkinsonism, Alzheimer's, declines in psychomotor speed and cognitive abilities. One change that is easily observed and consistent in mammals is the termination of the female reproductive cycle. Understanding the control and aging changes in the reproductive cycle may shed light on these maladies associated with aging.

The orchestration of changes in the brain, the pituitary and the ovaries maintains the rhythm of the reproductive cycle. Many factors point to catecholaminergic neuron activity as playing a key role in this organization. Alterations in the actvity of enzymes associated with the synthesis and degradation of the catecholamines is one possible source of changes in catecholaminergic neuron activity. The biosynthesis of the catecholamines involves the enzymes tyrosine hydroxylase, dihydroxyphenylalanine (DOPA) decarboxylase, dopamine B-hydroxylase, and phenylethanolamine N-methyltransferase. The two enzymes responsible for the degradation of the catecholamines are monoamine oxidase and catechol O-methyltransferase.

For my project I decided to explore the variation of DOPA decarboxylase activity during the estrous cycle and as a function of aging.

LITERATURE REVIEW

Many changes are associated with aging and nearly as many theories have been proposed to explain these changes. I will briefly mention a few of them, then concentrate on the neuroendocrine theories.

First, lipofuscin accumulation has been pointed to as a possible cause of aging. Lipofuscin, also called aging pigment, are brownish autofluorescent inclusion bodies which are thought to cause aging by impairing cell function. Toth (1968) found that they increase linearly with time in hepatocytes, myocardial cells, neurons, and other cells. Finch (1976) suggested that the rate of pigment accumulation may be associated with the lifespan of the animal since the accumulation in human myocardium (Strehler et al., 1959) is one fifth of that in the dog (Munnell and Getty, 1968). However, Finch (1976) also noted that there is no evidence of a functional deficit associated with pigment accumulation. Hayflick (1985) observed that Vitamin E deficiency increases lipofuscin deposition, but does not affect other age changes.

Another theory is the free radical theory. Free radicals are molecules which have an unpaired electron. They are very reactive and can react with stable molecules to form another free radical. This leads to a chain reaction which may result in the formation of lipofuscin (Chio and Tappel, 1969), the alteration of collagen, elastin,

and DNA (Harmon, 1981), and cause changes in mitochondria and lysosome membranes (Pryor, 1978). According to Segall and Sterrmberg (1988) catecholaminergic neurons, which show a decline in function with aging, contain high levels of free radicals.

There have also been proponents for the cross-linkage theory. Cross-linkages result when two or more molecules become linked by a covalent or hydrogen bond. An increase in cross-linkages with age has been found in collagen (Kohn, 1978; Hayflick, 1985) and DNA (Hayflick, 1985). However, it is not known if this cross-linking can cause aging.

One very popular theory is that cell loss causes changes that result in aging. Cell loss is not uniform throughout the brain, but is concentrated in certain regions or nuclei and depends upon the species and the indiviual. Hsu and Peng (1978) found neuron loss in the medial preoptic, anterior hypothalamic, and arcuate nuclei in rats. Cell loss may not be responsible for aging changes, because research has shown that losses of greater than 90 percent may be necessary in order for the effects of brain lesions to be seen (Heikkila et al., 1981).

There are also theories that suggest that changes in the DNA may induce aging. The somatic mutation theory made popular by Szilard (1959) and Failla (1958) proposed that mutations in somatic cells result in defective progeny. The error theory suggests that errors in DNA sequences are responsible for the production of faulty proteins, possibly enzymes which catalyze the wrong reactions leading to an "error catastrophe".

Another popular theory is the immunological theory. The body's

ability to produce antibodies, especially by the thymus-dependent system, decreases and autoimmunity increases with age (Hayflick, 1985). Changes in the immune system may not cause aging, but may be due to other aging changes, such as changes in the neuroendocrine system. The neuroendocrine system has been shown to affect the immune system.

The neuroendocrine theories of aging are usually divided into the neurotransmitter hypothesis, the pituitary hypothesis, the gonadal hypothesis and the stress hypothesis.

The neurotransmitter hypothesis suggests that changes in the levels of neurotransmitters are the cause of aging. The concentrations of the catecholamines, dopamine and norepinephrine, have been shown to decrease with age in the rat hypothalamus (Clemens & Meites, 1971; Quadri et al., 1973) Serotonin levels don't change significantly with age, but the balance between the serotonergic systems and the catecholaminergic systems is disturbed with age.

According to the pituitary hypothesis, the pituitary hormones are responsible for aging. Hypophysectomy retards aging of collagen, skeletal muscles, kidneys, heart, aorta, bones and gonads (Everitt & Burgess, 1976; Everitt, et al., 1983). However, many important pitutitary hormones can actually reduce the effects of aging when given to old rats.

The gonadal theory probably orginated when Brown-Sequard injected testicular extracts into old men, including himself, in 1889. The decrease in estrogen secretion in women and testosterone secretion in men does cause a decline in sexual function, general vigor,

and many other body functions, but it is not considered to be the primary cause of aging (Meites and Quadri, 1987). Gonadal steroids may actually cause nerve cell degeneration in the arcuate nucleus of the hypothalamus (Finch & Mobbs, 1983).

The stress theory proposes that environmental stresses lead to decline in the body's ability to adapt and maintain homeostasis (Selye & Tuchweber, 1976). Animals usually release adrenal cortical hormones in response to stress. Riegle (1983) showed a decrease in this response to stress with age.

Of these theories, the most popular is the neurotransmitter hypothesis. Reproductive decline is the most prominent change associated with aging, so the study of changes in the neuroendocrine control of the reproductive cycle may provide clues to the causes of aging.

The reproductive cycle (estrous cycle) in the female rat begins at about the age of forty days. It lasts 4-5 days and includes 4 stages--proestrus, estrus, metestrus and diestrus (Martin, 1976). These stages can be distinguished by vaginal cytology and by examination of the uterus and ovaries. During proestrus, which lasts 16-22 hours, the vaginal smears consist of many small epithelial cells and possibly a few leukocytes. The ovaries contain many follicles which have matured under the influence of follicle stimulating hormone. These follicles produce estrogen. The high estrogen levels cause the uteri to retain water and appear ballooned. During the 9-15 hours of estrus the epithelial cells become cornified or keratinized. They lose their round shape and their nuclei. Estrogen induces a surge

in luteinizing hormone levels. This surge may be mediated by the catecholamines which are thought to control the release of luteinizing hormone releasing hormone (Wise, 1985). The follicles ovulate due to the increasing levels of luteinizing hormone toward the end of estrus. Corpora lutea form from the ruptured follicles and begin to produce progesterone. The uteri are still ballooned at the beginning of estrus, but the increase in progesterone leads to the relaxation of the cervix. This allows the fluid to escape.

During metestrus, which lasts 10-14 hours, some leukocytes appear along with the cornified cells in the vaginal smear. This phase is usually during the night.

During diestrus, which may last 2-3 days, many leukocytes along with epithelial cells and sometimes cornified cells can be seen in the vaginal smears. The uteri are thin and cordlike. The corpora lutea continue to produce progesterone and there is a plateau and then a gradual decline.

After female rats reach 6 months of age, the percentage of rats which continue cycling begins to decline. More rats have five-day cycles. Then the cycles are replaced by either constant estrus or repeated pseudopregnancies. Some constant estrus rats then become repeatedly pseudopregnant (Aschheim, 1961). Constant estrus rats have vaginal smears which contain cornified cells. Ovulation does not take place, so there are no corpora lutea in the ovaries, hence progesterone levels are low (Lu et al, 1979). There are many follicles. Moderate amounts of estrogen are produced since there is no inhibition by progestogens (Aschheim, 1983). Thus the estrogen/progesterone

ratio is higher than in the young, cycling rat. Repeatedly pseudopregnant rats have diestrus vaginal smears. The ovaries contain large corpora lutea which produce progesterone. Progesterone concentration reaches a plateau at day 3 of pseudopregnancy. This plateau is lower than that found in young pseudopregnant controls (Fayein and Aschheim, 1980). The amount secreted is similar in old and young rats, but since old rats are larger, the concentration in the blood is lower (Ascheim, 1983). Estradiol levels are very low at diestrus (Lu et al., 1979). The low estrogen levels result in a lowered estrogen/progesterone ratio.

Could these changes in ovarian hormones bring about alterations in the estrous cycle or are these changes in response to changes in the control system? Aschheim (1983) attempted to answer this question by grafting the ovaries of old, constant estrous or repeated pseudopregnant rats into adult cycling rats. These rats resumed cycling. This suggests that the ovaries are not responsible for aging, but are affected by it.

The next level up in the control of the reproductive cycle is the pituitary. Much attention has focused on the control of the LH surge since its timing and amplitude is altered with age. Wise (1985) found that plasma LH concentrations in middle-aged (7-12 months) cycling rats peaked between 1600-1900 hours as compared to young cycling rats which had peak LH concentrations between 1600-1700 hours. She also found that peak LH concentrations were significantly lower in middle-aged rats than in young rats.

The release of LH from the pituitary is induced by the release of

LHRH from the hypothalamus. Levine and Ramirez (1982) observed LHRH release on the afternoon of proestrus which correlated with the secretion of LH. Fraser and McNeilly (1982) inhibited the LH surge by inactivating LHRH with antibodies.

Alterations in the LH surge with aging could be due to a decrease in the sensitivity of the pituitary to LHRH or to a decrease in the release of LHRH. Riegle and Miller (1978) found that 15 minutes after injecting LHRH serum LH did not increase as much in old rats as in young rats. Additionally, Peng and Huang (1972) showed that young rats resume cycling less frequently when grafted with pituitaries from old, acyclic rats than when grafted with pituitaries from young, cycling rats. These findings suggest that there may be a decrease in the pituitary's response to LHRH with age. However, changes in LHRH with age have also been found. Steger et al (1979) showed that hypothalamic LHRH concentrations are reduced in old, constant estrous rats yet they remain normal in old, repeated pseudopregnant rats. There are also changes in the release of LHRH with age. Many factors affect LHRH release. Miller and Cicero (1987) observed increases in *in vitro* LHRH release after treatment with ascorbate. Endogenous opioids inhibit LHRH secretion (Kalra and Kalra, 1983). Prostaglandin E2 increases blood LHRH levels (Ojeda et al., 1975). Oxytocin can inhibit LHRH release (Gambacciani et al., 1986). Estrogen and progesterone also stimulate LHRH release (Leadem and Kalra, 1984). Light and dark and pheromones may also affect LHRH release (Ramirez et al., 1984).

These factors may act directly on LHRH neurons or they may act indirectly through stimulation or inhibition of the catecholaminergic neurons or they may modify the effect of the catecholamines on the LHRH neurons. The effects of the catecholamines (dopamine, norepinephrine, and epinephrine) on LHRH release have been studied using a variety of methods. The mechanisms by which the catecholamines exert their control over LHRH release are quite complex and not fully understood.

Norepinephrine seems to play a stimulatory role in LHRH release. Increases in norepinephrine turnover in the arcuate nucleus (Rance et al., 1981) and the medial preoptic area (Honma and Wuttke, 1980) and increases in plasma norepinephrine levels (Nagle and Rosner, 1976) on the afternoon of proestrus implicate norepinephrine in the stimulation of LHRH. Some experimenters have induced LH surges by injection of norepinephrine (Kawakami and Ando, 1981; Tima and Flerko, 1974).

Inhibition of the LH surge by the alpha-adrenergic receptor blocker, phenoxybenzamine (Nagle and Rosner, 1980) suggests that norepinephrine and/or epinephrine act through alpha receptors to stimulate LHRH release. Leung et al. (1982) inhibited LH release by stimulating beta-receptors indicating norepinephrine and epinephrine may have different effects depending on which receptors are activated.

Not much data is available concerning the effects of epinephrine on the LH surge. Rubinstein et al. (1971) found that it induced ovulation in proestrus rats. Crowley and Terry (1981) inhibited the LH surge by using SKF64139 which inhibits epinephrine synthesis. This

evidence suggeststhat epinephrine is important for stimulation of LHRH.

The effect of the dopaminergic system on LHRH release is more confusing. Some evidence suggests a stimulatory role while other evidence contradicts this. Dopamine can increase LH levels even when a dopamine beta-hydroxylase inhibitor is used to prevent the conversion of dopamine to norepinephrine (Vijayan and McCann, 1987). Also the dopamine blocker, pimozide inhibits ovulation in proestrus rats (Clemens et al., 1977). However, Ramirez (1984) cites a publication in which pimozide increased LHRH release into hypophyseal portal blood. These conflicting results may be due to unknown effects of pimozide in addition to its action as a dopaminergic blocker . Data published by Rance et al. (1981) indirectly suggest an inhibitory role for dopamine. They found that the dopamine turnover rate decreased in the median eminence on the afternoon of proestrus. Yen (1980) found that dopamine infusion reduced LH levels in humans. Sarkar and Fink (1981) suggest that these different effects of dopamine may be due to the interaction of dopamine with different types of receptors.

There is evidence that not only do the levels of the catecholamines change with the reproductive cycle but also with aging. Decreases in norepinephrine concentrations have been found in the hypothalamus and medial basal hypothalamus of old rats (Simpkins et al., 1977). Estes and Simpkins (1981) observed decreases in norepinephrine concentrations in the arcuate nucleus, anterior hypothalamic nucleus, area retrochiasmatica and suprachiasmatic nucleus. Wise (1985) observed decreases in norepinephrine turnover

with age in cycling proestrus rats.

Decreases in dopamine concentration with age have been found in the hypothalamus (Estes and Simpkins, 1981) and more specifically in the medial basal hypothalamus (Simpkins et al., 1977) and discrete brain regions including the area retrochiasmatica, suprachiasmatic nucleus and the tuberoinfundibular dopaminergic projection to the neurointermediary lobes of the pituitary (Estes and Simpkins, 1981). However, in the arcuate nucleus dopamine concentrations in two year old female rats were not significantly different from young rats (Estes and Simpkins, 1981). Dopamine turnover decreased in the median eminence of female constant estrus rats (Demarest et al., 1981) and in the arcuate nucleus and several preoptic area nuclei in old female repeatedly pseudopregnant rats (Simpkins, 1983). However, dopamine turnover increased in the median eminence, ventromedial hypothalamic nucleus and in the tuberoinfundibular dopaminergic system of repeatedly pseudopregnant rats (Simpkins, 1983). Wise (1985) also found changes in the profile of dopamine turnover on the afternoon of proestrus with age in the arcuate nucleus. In young rats dopamine turnover was high between 1200 and 1400 hrs and decreased between 1500 and 1700 hrs on the afternoon of proestrus. In middle aged rats (7-12 mo.) which were still cycling the dopamine turnover did not decrease significantly between 1500 and 1700 hrs (Wise, 1985).

These changes in the catecholamines with age may be responsible for the changes in the sequence of events which lead to ovulation including the decline and decay of the LH surge. To test this

hypothesis some experiments have reinitiated estrous cycles using L-DOPA, the precurser of dopamine and norepinephrine (Quadri et al., 1973; Huang et al., 1976; Linnoila and Cooper, 1976).

If changes in the catecholamines are responsible for changes in the reproductive cycle with age, then the next step in elucidating the mechanisms involved in the aging of the reproductive cycle is to find the cause of change in the catecholamines. Cell loss or damage may be responsible for the decline in reproductive function with age. Peng (1983) reported loss of neurons in the arcuate, medial preoptic area, and ventromedial and lateral hypothalamic nuclei. Cell damage could be induced by estrogen (Brawer et al., 1978) or prolactin (Sarkar et al., 1984). It is possible that free radicals, cross linkages, and lipofuscin may injure, destroy or interfere with the function of neurons, but there is no evidence as yet to support this (Meites, 1988).

Alterations in the activity of the enzymes involved in the synthesis and degradation of the catecholamines could explain changes in their concentration.

The synthesis pathway of the catecholamines begins with tyrosine. It can be obtained through diet and can cross the blood brain barrier. Tyrosine hydroxylase in the presence of a pterin cofactor and catalase or ferrous ion converts tyrosine to L-dihydroxyphenylalanine (L-DOPA). L-DOPA is then decarboxylated by L-amino acid decarboxylase (AADC) or L-DOPA decarboxylase (DDC) to form dopamine. This reaction requires the cofactor pyridoxal phosphate. Then dopamine B-hydroxylase catalyzes the hydroxylation of dopamine to form norepinephrine. This process requires oxygen and an external

electron donor. Ascorbate acts as the reducing agent. Norepinephrine can then be methylated to form epinephrine. The enzyme involved in this reaction is phenylethanolamine N-methyltransferase. S-adenosylmethionine acts as the methyl group donor.

Two enzymes monoamine oxidase (MAO) and catechol-O-methyltransferase are largely responsible for the degradation of the catecholamines.

In some studies attempts were made to correlate the activity of the enzymes involved in the synthesis and degradation of the catecholamines with stages of the estrous cycle.

Carr and Voogt (1980) found a decrease in tyrosine hydroxylase activity at 1200 hrs on proestrus and increases in dopamine B-hydroxylase activity at 1400 hrs on diestrus and at 1200 hrs on proestrus in the medial basal hypothalamus.They interpreted these results as a reflection of decreased dopamine synthesis and increased norepinephrine synthesis at these times. Pasqualini et al. (1988) also noted a decrease in tyrosine hydroxylase activity at 1200 hrs as well as an increase in the afternoon of proestrus in the tuberoinfundibular dopaminergic system.

Kamberi and Kobayashi (1970) reported an increase in hypothalamic monoamine oxidase activity at 1000 hrs in proestrus and 1500 hrs in estrus. They suspected that this increase in monoamine oxidase activity would lead to a reduction in one of its substrates which would stimulate the secretion of lutenizing hormone. Salseduc et al. (1966) reported increases in catechol-O-methyltransferase activity on proestrus compared to diestrus. This could have the same

effect as the increases in monoamine oxidase activity.

Changes in the hypothalamic activities of tyrosine hydroxylase, dopamine B-hydroxylase and monoamine oxidase with age have also been reported.

Reis et al. (1977) found increases in tyrosine hydroxylase and decreases in dopamine B-hydroxylase activity with age in the hypothalamus. These changes directly oppose the changes found in proestrus. Bhaskaran and Radha (1984) reported increases in monoamine oxidase with age in the hypothalamus.

Most of these studies have determined enzyme activities in whole brain or large regions of the brain such as the hypothalamus, medial basal hypothalamus, cerebral cortex, and hindbrain. I thought that it would be more revealing to study discrete brain regions using the micropunching method described by Palkovits and Brownstein (1988). I decided to take tissue samples from the medial preoptic area and the arcuate nucleus since both of these areas are involved with the control of luteinizing hormone. The preoptic area has been implicated in the cyclic release of the gonadotropins (Ramirez et al, 1984). Brain lesions in the medial preoptic area can induce repeated pseudopregnancies in young rats (Clemens and Bennett, 1977). The arcuate nucleus is implicated in the tonic release of luteinizing hormone releasing hormone (Aschheim, 19).It is located on the pathway between the medial preoptic area and the median eminence - the site of LHRH release into the hypophyseal portal system. Lesions in the arcuate nucleus prevent ovulation in rhesus macaques (Plant et al.,1978).

DOPA Decarboxylase

As discussed before, there are six enzymes involved in the synthesis and degradation of the catecholamines. I decided to investigate changes in dopa decarboxylase activity in the medial preoptic area and the arcuate nucleus on the day of proestrus and with age.

Aromatic L-amino acid decarboxylase (EC 4.1.1.28) (AADC) catalyzes the decarboxylation of L-amino acids including L-B-3,4-dihydroxyphenylalanine (L-dopa), 5-hydroxytryptophan (5-HTP)--phenylalanine, trptophan and tryrosine. AADC activity has been found in various mammalian tissues and serum, human liver and brain (Lee, 1986). It also is found in pancreatic islets, but the physiological significance of this is not known (Lindstrom, 1986). In the central nervous system, the activity varies widely, being highest in the neostriatium, followed by the hypothalmus, the septum, the brainstem, the thalamus, the piriform lobe, spinal cord, and hippocampus, with the frontal cortex having the lowest activity (Bouchard et al., 1981).

Dopa decarboxylase (DDC) and 5-HTP decarboxylase (5-HTPDC) were originally thought to be two separate enzymes. However, Christenson et al. (1970) found after purification that a single homogenous protein was capable of decarboxylating both L-Dopa and 5-HTP as well as other aromatic amino acids.

Streffer found in 1967 that L-Dopa and 5-HTP are competitive inhibitors for one another. Rahman and Nagatsu suggested in 1981 that there may be different forms of AADC originating from different tissues having different substrate specificity, after finding a significant difference in developmental patterns in rat serum taken from rats 1 day old to 15 weeks old. In other words, they suggest different isozymes of AADC may be produced at different ages in the rat. Isozymes are groups of enzymes that are very similar in catalytic properties, but may be differentiated by variations in physical properties, such as electrophoretic mobility or isoelectric point. Cavalli-Sforza et al. (1974) reported that within a species there seems to be neither tissue nor strain difference in electrophoretic mobility of AADC. However, they found that the electrophoretic mobility of the enzyme differs among various species. Park and co-workers presented findings in 1986 which support these observations. They found that rat brain and rat adrenal tissue had identical isoelectric points while bovine adrenal tissue had a lower isoelectric point meaning that the pH at which rat AADC is least soluble is different from the pH at which bovine AADC is least soluble. The isoelectric point (pI) is the position that a protein molecule migrates to in a gel support subjected to an electric field in which a pH gradient has first been formed. This point is the location of the pH at which the protein is least soluble, the molecule has no net charge and fails to move in the electric field. Despite species differences in pI, the primary structure of AADC from different species is thought to be similar, with post translational modifications accounting for the

different charge forms.

Ichinose et al. (1985) found that an inhibitor of AADC seems to be present in human tumor tissue which may modify the substrate specificity of the crude enzyme.

The Rahman group concluded in 1984 that the presence of endogenous inhibitors in monkey serum affected AADC activities for DOPA & 5-HTP differently to change the ratio between DDC & 5-HTP DC activities of a single AADC. Studies showing different ratios of activity for the different substrates have been thought to indicate two types of AADC but may indicate the presence of inhibitors which affect AADC activities for L-DOPA and 5-HTP differently.

Since we were interested in finding the activity of the enzyme responsible for the decarboxylation of L-DOPA in this assay, I used L-DOPA as the substrate and optimized the assay for the decarboxylation of L-DOPA and determined the activity by the amount of the product, dopamine, formed. Punches for the assay of DOPA decarboxylase were homogenized in 200ul 0.32M sucrose. Some groups homogenize tissue in different media such as 0.32M or 0.25M sucrose (Rahman et al., 1982; Rahman et al., 1981; Bouchard et al., 1981; Nagatsu et al., 1979) or 0.05M Tris-HCl buffer pH 6.0 (Lamprecht & Coyle, 1972) or water (Lin et al., 1984). From this homogenate two 25ul aliquots were taken - one for the enzyme assay and one for the blank - and 10ul was used for the determination of protein content using the Pierce BCA protein assay. Nagatsu et al. (1979) and Sims et al. (1973) used the Lowry method (1951), and most other investigators (Siow & Dakshinamurti, 1985,1986; Ichinose et al., 1985; Bouchard et

al., 1981; Nagatsu et al., 1985; Rahman et al., 1984) used BioRad protein assay based on the method of Bradford (1976). However, I used the Pierce BCA assay based on the method of Smith et al. (1985) because of its simplicity and sensitivity.

The blank is used to check for the amount of dopamine in the tissue not formed by the chemical reaction catalyzed by AADC during the incubation. I used a blank which did not include the substrate. Sims et al. (1973) used heat-killed homogenate for the blank. Lamprecht & Coyle (1972) use 0.1mM K-486 which is a specific inhibitor of dopa decarboxylase. Other investigators have used D-DOPA instead of L-DOPA (Rahman et al. 1981a,b,c; Nagatsu, 1979 & 1985; Inchinose 1985. According to Nagatsu (1979), the non-enzymatic reaction can be completely cancelled by a control with D-dopa as substrate. This probably would have been the best blank to use, but in the blank that I used we found no dopamine.

I then added 10 ul of 0.05mM pargyline and 0.05mM pyridoxal-phosphate solution and 10 ul of 0.375 M sodium phospate buffer to the enzyme and the blank solution.

Sims et al. (1973) and Schott & Clark (1952) found that preincubation of the tissue homogenate with pyridoxal-phosphate was essential for obtaining maximal rates of dopa-decarboxylase activtity. Also homogenization and storage of the tissue sample in pyridoxal-phospate resulted in better preservation of enzymic activity than when tissue was homogenized in water. Tissue homogenates in pyridoxal-phospate which were frozen and thawed once showed increases in dopa decarboxylase activity (Sims et al., 1973).

Bouchard et al. (1981) found endogenous pyridoxal-phospate sufficient to permit the decarboxylation of L-DOPA, but they found that the addition of 2.5 mM pyridoxal-phospate doubled the activity of DDC in the brainstem and increased it by 2.5 fold in the liver. However, increasing the pyridoxal phosphate concentration over 20mM led to a progressive and significant decrease in DDC activity in the brainstem and liver, respectively. (Bouchard et al., 1981) This inhibition of AADC with high concentrations of pyridoxal phosphate is said to be due to the formation of a Schiff base. (Ichinose et al., 1985) Christenson et al. (1970) attribute the decrease in DDC activities in the presence of high concentrations of pyridoxal-phosphate to the formation of the tetrahydroisoquinoline cyclization product. Bouchard et al. (1981) and Lee et al. (1986) found maximum activity with a concentration of 0.01-0.2 mM pyridoxal phosphate.

Pargyline is a monoamine oxidase inhibitor which prevents the oxidation of dopamine and has no effect on DOPA decarboxylase activity at concentrations up to 3 mM (Bouchard et al., 1981). Sims et al. (1973) used ascorbate to prevent oxidation, but most recent papers use pargyline. Pargyline has been shown not to affect decarboxylase activity in the brainstem of cats in concentrations up to 3M. In contrast to other monoamine oxidase inhibitors such as iproniazide phosphate and harmaline which decrease decarboxylase activity in the brainstem and to a greater extent in the liver. A concentration of 500 uM or more was necessary to decrease DDC activity in the brainstem (Bouchard et al., 1981).

The buffer solution used here is a sodium phosphate buffer at

pH 6.6. Most investigators have used sodium phosphate buffer (Lamprecht and Coyle, 1972; Nagatsu, 1979,1985; Ichinose, 1985; Bouchard, 1981; Rahman, 1981a,b,c,1984). Sodium imidazole is the buffer used by Sims et al. (1973). Sims et al. (1973) found a fall in activity with time of incubation which was greater in the phosphate buffer than in the imidazole buffer.

According to Sims et al. (1973) the optimum pH for the activity of DOPA-decarboxylase is 6.8+0.1. Others have used a pH of 7.2 (Nagatsu, 1979; Nagatsu, 1985; Ichinose, 1985; Rahman, 1981a,b,c,1984). Bouchard (1981) used a pH 8.0, Lin et al. (1984) used pH of 6.6, and Lee (1986) used a pH of 6.8. Rahman (1980) reported an optimum pH at 7.2 for DOPA-decarboxylase. The use of an alkaline pH is puzzling since the substrate, DOPA, and the reaction product dopamine are unstable at alkaline pH (Christenson, 1970).

I then added 10 ul of 4mM L-DOPA to the enzyme solution, but not to the blank. Preliminary experiments determined the concentration of 4mM L-DOPA to be the optimum concentration. Higher concentrations did not result in increased production of dopamine, but increased interference with the HPLC analysis. L-DOPA is the substrate for AADC and its addition initiates the reaction. This reaction mixture is then incubated at 38 degrees C for 30 minutes. Thirty-eight degrees celsius was found to be the optimum temperature for DOPA decarboxylase activity (Sims et al., 1973).

Dopa decarboxylase activity has been reported to be linear up to 30 minutes at 38 degrees C (Sims et al. 1973). Lee (1986) reported linear activity up to 70 minutes at 37 degrees C. They recommend a

30-minute incubation time.

The reaction is stopped by immersing the solutions in boiling water for 45 seconds. Sims et al. (1973) and Lin et al. (1984) stop the reaction by immersing the sample in boiling water for 45 seconds. Lamprecht & Coyle (1972), Nagatsu (1979), Lee et al. (1986), Rahman et al. (1981,1982,1984), used 3M trichloroacetic acid. Lindstrom (1986) used 0.1M HCl.

The sample is centrifuged. Internal standard is added. The supernatant is then analyzed using HPLC.

METHODOLOGY

ANIMALS

For this experiment, I used young, regularly cycling and old, pseudopregnant female Sprague-Dawley rats (Amitech Inc., Omaha, NE). After arrival in our lab the animals were allowed to rest for at least eight days. The animals were housed in air conditioned rooms and provided water and feed *ad libitum*. The lights were turned on at 0700 hours and off at 1900 hours. The average age of the young rats was 4.3 $\pm$ 0.4 (S.D) months and the average age of the old rats was12.5 $\pm$ 0.1 months. I determined the stage of the estrous cycle or pseudopregnancy by vaginal smears taken through at least two full estrous cycles or ten days. On the day of proestrus, I killed by decapitation, four to ten rats at each of the following times: 1000, 1200, 1400, 1600, 1800 and 2000 hours. I also killed six young rats at 1000 hours during diestrus and six old rats at 1000 hours during pseudopregnancy on days that they had leukocytic smears. After killing the rats and quickly removing the brains, I checked the uterus and the ovaries to verify the stage of the cycle.

MICRODISSECTION

After killing the rats, I removed the brains within two minutes and mounted them on specimen holders using Tissue-Tek (Miles, Inc., Elkhart, IN) and dry ice. I wrapped the brains in plastic wrap and placed them in a cryostat (SLEE, Pittsburgh, PA) and allowed them to equilibrate for 8-24 hours to its temperature between -10 and -12 degrees C. I then sliced sections approximately 300 microns thick and collected them on cover glasses. I placed the cover slips on a Flexi-cool cold stage (FTS Systems, Stoneridge, NY) which maintains a temperature of -10 degrees C. I located the medial preoptic area and the arcuate nucleus using a stereotaxic atlas (Paxinos & Watson, 198). I then used 500 and 750 micron cannnulae (Zivic-Miller, Allison Park, PA) to obtain tissue punches from the medial preoptic area and the arcuate nucleus. I took two to four punches of medial preoptic area and three to eight punches of arcuate nucleus. I then stored these punches in 200 ul 0.32M sucrose solution at - 60 degrees C until I was prepared to assay the activity of DOPA decaboxylase.

DOPA DECARBOXYLASE ASSAY

I removed the samples from the freezer and immediately sonicated them using a Kontes (Vineland, N.J.) micro ultrasonic cell disrupter at an output setting of 50 until they were thawed (30 seconds). I then transferred 25 ul of this solution into each of two 1.5

ml microsample tubes (Dew Scientific, Columbus, OH)--one for the enzyme assay and one for the blank assay. I added 400 ul of milli-Q (Millipore, Bedford, Mass.) water to the enzyme and 410 ul of 0.75 M phosphate buffer pH 6.6 and 10 ul of 1mM pargyline in I mM pyridoxal-phosphate to both the enzyme and the blank. I initiated the reaction by adding 10 ul of 4 mM L-Dopa to the enzyme mixture, but not to the blank. I then incubated the enzyme and the blank in a Dubnoff Metabolic Shaking Incubator (Precision Scientific, Chicago, IL) which was not shaking. I kept the samples at 38 degrees C for 30 minutes. I stopped the reaction by immersing the samples in boiling water for 45 seconds. I then centrifuged the samples for 10 minutes in an IEC Clinical Centrifuge (Needham, Hts., Mass.) at room temperature and a speed of 7. There was no visible sediment because the amount of tissue was so small. If I could analyze the samples at that time, I immediately injected them into the HPLC system. Otherwise, I kept them in a Kelvinator (Manitowoc, Wis.) Series 500 ultra-freezer at -70 degrees C until I was prepared to analyze them.

DOPA DECARBOXYLASE ASSAY SOLUTIONS

A. 1mM pargyline (F.W. 195.7 g/mole) in 1mM pyridoxal-phospate (F.W. 247.1 g/mole

0.0096 g pargyline and 0.0025 g pyridoxal-phospate

Add Milli-Q water to 10 ml

B. 0.75 M phosphate buffer pH 6.6

10.3493 g monbasic sodium phosphate (F.W. 137.99 g/mole)

Add Milli-Q water to make 90 ml

Add sodium hydroxide to pH 6.6

Add milli-Q water to 100 ml

C. 4 mM L-Dopa (F.W. 197.19 g/mole)

0.0789 g L-dopa

Add Milli-Q water to 100 ml

Stir until L-Dopa goes into solution

ASSAY

	Enzyme	Blank
Sample	25 ul	25 ul
milli-Q water	400 ul	410 ul
pargyline	10 ul	10 ul
pyridoxal-P		
phospate buffer	10 ul	10 ul
L-Dopa	10 ul	--
Total volume	455 ul	455 ul

HPLC-EC

I thawed samples that were frozen by holding them in my hand for about a minute--until completely thawed. I then analyzed them using High Performance Liquid Chromatography with Electrochemical Detection. Our system included a Shimadzu (Columbia, MO) LC-6A solvent delivery module; a Rheodyne (Cotati, CA) injector; a BAS (West Lafayette, IN) Biophase ODS, 30 x 4.6 mm, 5 um guard column; a BAS Phase-II ODS, 250 x 4.6 mm, 5um reverse phase analytical column; a BAS LC-4A amperometric detector and a Shimadzu C-R6A Chromatopac integrator. I made the mobile phase according to the following recipe.

MOBILE PHASE

1) Fill a one-liter graduated cylinder approximately half full with Milli-Q water.

2) Add 35 ml of acetonitrile (Fisher, Fair Lawn, N.J.) to the Milli-Q water.

3) To the pyrogen-free water also add the following ingredients:

A. 14.15g monochloroacetic acid (also known as chloroacetic acid) (Sigma, St. Louis, MO).

B. 4.67g sodium hydroxide (Fisher, Fair Lawn, N.J.)

C. 0.300g 1-octanesulfonic acid sodium salt (also known as OSA).

D. 0.250g ethylnediaminetetraacetic acid (disodium salt) more commonly referred to as EDTA (Mallinckrodt, St. Louis, MO).

4) Add Milli-Q water to make a one-liter solution.

5) Stir until all the chemicals are in solution.

6) Filter through a 0.22 micron pore size filter (Millipore, Bedford, Mass.) into a Kontes (Vineland, N.J.) Ultra-ware HPLC reservoir which contained 25 ml tetrahydrofuran (Fisher, Fair Lawn, NJ).

The solvent delivery module pumped the mobile phase at a constant flow rate of 1.7 ml/min. The detector maintained a potential of +0.80 volts.

I made concentrated and dilute standards according to the following recipes.

Dopamine (DA) - concentrated

11.70 mg Dopamine hydrochloride (Sigma, St. Louis, MO) in 100 ml of 0.1 M perchloric acid

3,4-Dihydroxybenzylamine (DHBA) - concentrated

14.96 mg 3, 4-Dihydroxybenzylamine hydrobromide (Sigma, St. Louis, MO) in 100 ml of 0.1 M perchloric acid

DHBA - dilute

25 ul DHBA concentrated in 100 ml of 0.1m perchloric acid.

DA/DHBA - dilute

25 ul DHBA Concentrated and 25 ul DA concentrated in 100 ml of 0.1 M perchloric acid.

The dilute standards contained 23.63 pg/ul of DHBA and DA.

I injected 10 ul of the DA/DHBA dilute standard containing 236.3 pg of both DA and DHBA. Then I mixed 10 ul of dilute DHBA with 25 ul of sample. I used DHBA as an internal standard and I calculated the sample concentration of dopamine using the absolute calibration curve method according to the Shimadzu Chromatopac C-R6A instruction manual. The formula is

$$\text{Content} = \frac{\text{Area of dopamine (sample)} \times \text{weight of dopamine (std)}}{\text{Area of dopamine (std)}}$$

Protein Assay

In order to determine the protein concentration, I used the Pierce (Rockford, IL) BCA Protein Assay Reagent. I made standards using bovine serum albumin (BSA)(Sigma, St. Louis, MO) and 0.32 m sucrose solution.

I first made a stock solution containing 2 mg BSA/ml sucrose. I then made a set of standards according to the chart below.

Volume of BSA (2mg/ml)	Volume of Sucrose	Final Conc. of Calibration Std
0.05 ml	1.95 ml	50 ug/ml
0.10 ml	1.90 ml	100 ug/ml
0.15 ml	1.85 ml	150 ug/ml
0.20 ml	1.80 ml	200 ug/ml
0.25 ml	1.75 ml	250 ug/ml

I transferred 100 ul of each of the standards to labeled 12 x 75 mm test tubes. I transferred 10 ul of sample and 90 ul of sucrose to labeled 12 x 75 mm test tubes. In one test tube labeled blank I put 100 ul of sucrose.

standards	samples	blank
100 ul	10 ul sample + 90 ul sucrose	100 ul sucrose

I then added 2.0 ml of the Pierce BCA working reagent solution which was made up of 100 parts Reagent A and 2 parts Reagent B.

Reagent A contains sodium carbonate, sodium bicarbonate, BCA detection reagent and sodium tartrate in 0.2 N NaOH. Reagent B contains 46 copper sulfate solution. I then incubated these samples for 30 minutes at 52 degrees C in the Dubnoff Metabolic Shaking Incubator (Precision Scientific, Chicago, IL). I allowed the tubes to cool to room temperature before measuring the absorbance of each tube at 562 nm vs. a water reference. I used a Coleman (Maywood, IL) Junior II Spectophotometer to measure the absorbance. I subtracted the value of the blank from the value found for the standards or the samples. I prepared a standard curve by plotting the absorbance at 562 nm vs. the protein concentration of the standards. I used this curve to determine the protein concentration of the unknown protein samples.

FINAL CALCULATIONS

I multiplied the quantity of dopamine that I got by 14.2 since that was the portion of the total sample that I injected into the HPLC system. I then divided this number by the amount of protein in each sample and by 30 (minutes incubation). I reported the results in terms of picomoles dopamine/mg protein/minute incubation.

STATISTICS

I compared each of the different groups using the student's T test.

RESULTS :

DOPA decarboxylase activity in the Arcuate nucleus on the day of proestrus.

Dopa decarboxylase activity in the Arcuate nucleus on the day of proestrus is shown in figure 1. It was 90 ± 33 (mean ± SE) pmoles/ mg protein/ min at 1000 hrs and remained at this level through 1200 hrs. Then decreased by nearly 50 percent to 50 ± 8 pmoles/mg protein/min at 1400 hrs. This was significantly different from 1200 hrs ($p = 0.064$). After that it gradually increased and by 2000 hrs it was over 300 percent of the activity at 1400 hrs. The difference between the activity at 1400 hrs and the activity at 2000 hrs was significant ($p < 0.05$).

DOPA decarboxylase activity in medial preoptic area on the day of proestrus

DOPA decarboxylase activity on the day of proestrus in the medial preoptic area is shown in figure 2. It was low 42 ± 13 pmoles/mg protein/min at 1000 hrs. By 1200 hrs it increased by over 300 percent to 134 ± 29 pmoles/mg protein/ min. The difference between these two levels was significant ($P< 0.05$). After 1200 hrs there was a slight decrease in DOPA decarboxylase activity to 105 ± 22 at 1400 hrs. This level was essentially maintained through 2000

hrs with minor fluctuations. These values were not significantly different from 1200 hrs although they were all higher than those at 1000 hrs and significantly so at 1400 hrs ($P < 0.05$).

Comparison of DOPA decarboxylase activities at 1000 hrs on proestrus and on diestrus.

Figures 3 and 4 show DOPA decarboxylase activity at 1000 hrs on proestrus and diestrus in the arcuate nucleus and in the medial preoptic area, respectively. In both of these regions the activity was significantly higher in diestrus than on proestrus. The activity in the arcuate on diestrus was 325 ± 113 pmoles/mg protein/min. This was nearly four times as much activity as on proestrus at the same time ($p < 0.05$). In the medial preoptic area the activity was 167 ± 60 pmoles/mg protein/min on diestrus. Again this was about four times the activity on proestrus ($p = 0.069$).

Comparison of DOPA decarboxylase activity in young diestrus and old pseudopregnant animals

Figure 5 shows the DOPA decarboxylase activity in the arcuate nucleus of young diestrus rats and old pseudopregnant rats. Data from one old pseudopregnant rat was ommitted since its activity was 14 times the mean of the other values. Without this value the activity of the old pseudopregnant rats was 50 ± 20 pmoles/mg protein /min and the difference between the old and young was significant ($p < 0.05$). With this value the activity of the old group was 161 ± 112 pmoles/

mg protein/ min and the difference between the old and young was not significant (P = 0.32).

DOPA decarboxylase activity in the medial preoptic area of young diestrus and old pseudopregnant rats is shown in figure 6. It was 32 ± 15moles/mg protein/min in the old rats. This was approximately one fourth of the activity in the young rats (p = 0.054).

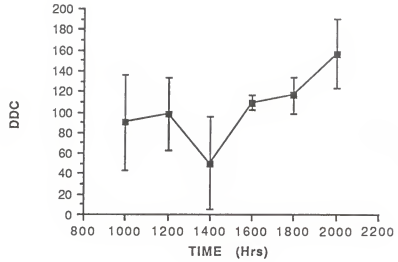

Figure 1. DOPA decarboxylase (DDC) activity (picomoles dopamine/mg protein/minute) in the arcuate nucleus at various times during proestrus.

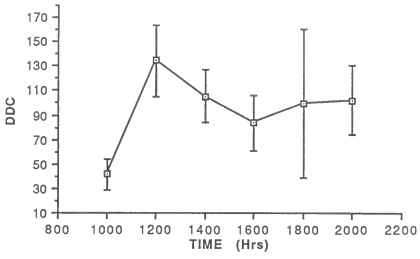

Figure 2. DOPA decarboxylase (DDC) activity (picomoles dopamine/mg protein/minute) in the medial preoptic area at various times during proestrous.

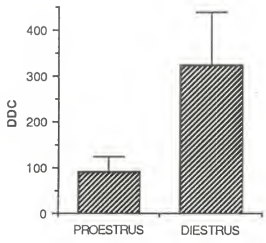

Figure 3. DOPA decarboxylase (DDC) activity (picomoles dopamine/mg protein/minute) in the arcuate nucleus at 1000 hours in proestrous and diestrous rats.

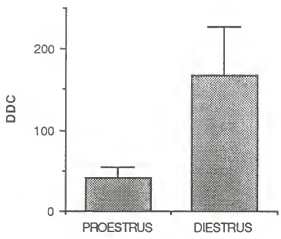

Figure 4. DOPA decarboxylase (DDC) activity (picomoles dopamine/mg protein/minute) in the medial preoptic area (MPA) at 1000 hours in proestrous and diestrous rats.

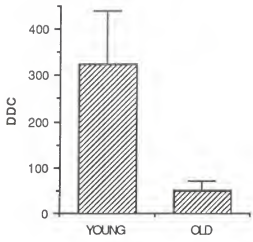

Figure 5. DOPA decarboxylase (DDC) (picomoles dopamine/mg protein/minute) activity in the arcuate nucleus at 1000 hours in young, diestrous and old, pseudopregnant rats.

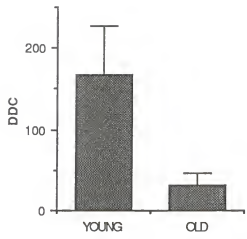

Figure 6. DOPA decarboxylase (DDC) activity (picomoles dopamine/mg proein/minute) in the medial preoptic area (MPA) at 1000 hours in young, diestrous and old, pseudopregnant rats.

DISCUSSION

Our results suggest changes in DOPA decarboxylase activity in the medial preoptic area and the arcuate nucleus do correlate with changes in the estrous cycle and aging. The signifigance of this will become more clear when data concerning changes in the other enzymes associated with the synthesis and degradation of the catecholamines in these areas are available.

Changes in DOPA decarboxylase activity during proestrus

To my knowledge no one has correlated changes in DOPA decarboxylase activity with various stages of the estrous cycle. However, Carr and Voogt (1980) found a decrease in tyrosine hydroxylase activity and an increase in dopamine B-hydroxylase in the medial basal hypothalamus which includes the arcuate nucleus at 1200 hours. Their lighting schedule was different than ours (light hours - 0400 to 1600). They found changes eight hours after the lights came on. Thus on our lighting schedule (light hours - 0700 to 1900) the changes would take place at 1500 hours. This is nearer to the time that I found decreases in DOPA decarboxylase activity in the arcuate nucleus (1400 hours). Pasqualini et al. (1988) also found changes in tyrosine hydroxylase activity during the estrous cycle. They found decreases in tyrosine hydroxylase activity between 1200 and 1500 hours and then an increase at 1700 hours that disappeared by 1800 hours. Carr and Voogt (1980) suggest the decreases in

tyrosine hydroxylase activity are responsible for decreases in dopamine production. Decreases in dopa decarboxylase activity would also have this effect. Kerdelhue et al. (1989) found dopamine levels in the median eminence remained stable during proestrus, but dihydroxyphenylacetic acid (DOPAC - the major dopamine metabolite) levels decreased on the afternoon of proestrus. Rance et al. (1981) found decreases in dopamine turnover in the median eminence during the afternoon of proestrus. These changes in DOPAC levels and in dopamine turnover are indications of decreases in dopamine release. Since the tuberoinfundibular dopaminergic system originates in the arcuate nucleus and leads to the median eminence (Palkovits, 1981), changes in dopamine release in the median eminence could be a result of a decline in dopa decarboxylase activity in the arcuate nucleus. Decreases in dopa decarboxylase activity could be a result of increases in estrogen which has been shown to inhibit dopamine release into the hypophyseal portal system (Cramer et al., 1979).

The dopa decarboxylase activity in the medial preoptic area was increased in the afternoon as compared with the activity at 1000 hours. The signifigance of this increase is not clear. There is no information available on enzyme activities in the medial preoptic area. The medial preoptic area is innervated by both noradrenergic and dopaminergic fibers (Palkovits, 1981) and the increase in dopa decarboxylase activity in the afternoon of proestrus could lead to an increase in norepinephrine synthesis.

Comparison of DOPA decarboxylase activity in proestrous and diestrous rats

DOPA decarboxylase activity in diestrous rats at 1000 hours was signifigantly higher than in proestrous rats at the same time in both the medial preoptic area and the arcuate nucleus. No one has made this comparison before and the signifigance is unclear. Carr and Voogt (1980) determined tyrosine hydoxylase and dopamine B-hydroxylase activities on diestrus and proestrus, but here again their lighting schedule was three hours earlier than ours. If we adjust our time to theirs the difference we found would have occurred at 0700 hours, a time when they took no measurements.

Comparison of DOPA decarboxylase activity in young, diestrous and old, pseudopregnant rats.

We found signifigant decreases in both the medial preoptic area and the arcuate nucleus of old, repeatedly pseudopregnant rats as compared with young, diestrous rats. In contrast to our results Finch (1973) found no signifigant changes in DOPA decarboxylase activity in mouse hypothalamus and striatum with age. Reis et al. (1977) found no signifigant changes in the caudate nucleus of old male rats. Harik and McCracken (1986) found no signifigant changes in the cerebral cortex of old male rats. These discrepancies may be due to species or sex differences. Decreases in dopa decarboxylase activity could be responsible for the decreases in dopamine and norepinephrine found

with age. According to Porter et al. (1985) decreases in tyrosine hydroxylase activity and not DOPA decarboxylase activity are responsible for decreases in dopamine release in portal blood in old rats. They mention that Reymond et al. found decreases in tyrosine hydroxylase activity in the medial basal hypothalamus of aged rats. Demarest et al. (1981) observed decreases in DOPA accumulation in the median eminence after treatment with a DOPA decarboxylase inhibitor in old, constant estrus and repeatedly pseudopregnant rats indicating a decrease in tyrosine hydroxylase activty with age. However, Reis et al. (1977) found signifigant increases in tyrosine hydroxylase activity in the hypothalamus and Harik and McCracken (1986) found increases in tyrosine hydroxylase activity in the cerebral cortex of old, male rats . If there are similar increases in old, female rats it could be due to a lack of negative feedback caused by decreases in catecholamine production as a result of a decline in dopa decarboxylase activity.

Conclusion

DOPA decarboxylase activity changes during the estrous cycle and with aging. DOPA decarboxylase activity decreases in the arcuate nucleus and increases in the medial preoptic area on the afternoon of proestrus. DOPA decarboxylase activity in both of these areas is greater in diestrus than proestrus at 1000 hours. With age, the activity in both of these areas decreases. The signifigance of these results is not known, but this information does add to our knowledge and may provide important clues when the activities of the other enzymes associated with the synthesis and degradation of the catecholamines in these areas becomes available.

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APPENDIX

TABLE 1

DOPA decarboxylase activity (picomoles dopamine/mg protein/min) in the Arcuate Nucleus of Young Cycling Rats at Various Times on the Day of Proestrus.

Time(hrs)	1000	1200	1400	1600	1600	2000
No. of Rats	7	10	6	4	5	6
	69	101	39	243	206	62
	48	43	64	66	99	92
	27	85	28	68	44	61
	173	60	52	57	190	124
	41	70	38		40	318
	19	164	78			279
	253	103				
		126				
		201				
		18				
Mean ± SE	90	98	50	109	116	156
	±	±	±	±	±	±
	33	18	8	45	35	46

TABLE 2

Dopa Decarboxylase Activity (picomoles dopamine/mg protein/min) in the Medial Preoptic Nucleus of Young Cycling Rats at Various Times on the Day of Proestrus.

TIME	1000	1200	1400	1600	1800	2000
No. of rats	6	10	6	6	4	5
	84	106	79	42	268	66
	56	109	180	91	107	74
	11	44	96	86	14	146
	8	88	158	188	10	188
	25	130	38	40		35
	66	120	82	55		
		212				
		364				
		57				
		111				
MEAN	42	134	105	84	100	102
±	±	±	±	±	±	±
SE	13	29	22	23	60	28

TABLE 3

DOPA Decarboxylase Activity (picomoles dopamine/mg protein/min) in the Arcuate Nucleus at 1000 Hours in young Diestrous and old Repeatedly Pseudopregnant Rats

	Young	Old
No. of Rats	5	5
	671	28
	92	17
	222	130
	509	38
	133	38
Mean	325	50
±	±	±
S.E.	113	20

TABLE 4

DOPA Decarboxylase Activity (picomoles dopamine/mg protein/min) in the Medial Preoptic Area at 1000 Hours in young Diestrous and old Repeatedly Pseudopregnant Rats

	Young	Old
No. of Rats	6	6
	66	9
	166	84
	46	75
	82	21
	441	0
	202	3
Mean	167	32
±	±	±
SE	60	15

THE EFFECTS OF THE ESTROUS CYCLE AND AGING ON DOPA-DECARBOXYLASE ACTIVITY IN HYPOTHALAMIC NUCLEI

by

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The purpose of this study was two fold: 1) to determine dihydroxyphenylalanine (DOPA) decarboxylase activity during the estrous cycle and 2) to investigate if there are any changes in DOPA decarboxylase activity with aging. I used two groups of female Sprague-Dawley rats - young (4.3 ± 0.4 months S.D.) and old (12.5 ± 0.1 months). I took vaginal smears for a minimum of eight days (two normal cycles) to determine the stage of their cycle. I then decapitated the young rats at 1000, 1200, 1400, 1600, 1800 and 2000 hours during proestrus and at 1000 hours on diestrus. I killed the old rats at 1000 hours after they were determined repeatedly pseudopregnant. I then verified their reproductive status by examination of the uteri and the ovaries. Microcannulae were used to remove tissue from the medial preoptic area and the arcuate nucleus. The sample size was 500-750um in diameter and 300um in thickness. I determined DOPA decarboxylase activity in these samples by measuring the end product, dopamine, using high performance liquid chromatography. DOPA decarboxylase activity in the medial preoptic area was low at 1000 hours, increased signifigantly to 134 ± 29 (S.E.) picomoles dopamine/mg protein/minute at 1200 hours and remained high throughout the afternoon, signifigantly so at 1400 hours ($p<0.05$). At 1000 hours the DOPA decarboxylase activity in the arcuate nucleus was 90 ± 33 picomoles dopamine/mg protein/minute. It remained at this level through 1200 hours, then decreased by nearly 50% at 1400

hours. After that, it steadily increased and was signifigant at 2000 hours ($p<0.05$). In both the medial preoptic area and the arcuate nucleus, the activity of DOPA decarboxylase was higher on diestrus than on proestrus (arcuate nucleus $p<0.05$, medial preoptic area $p=0.069$). The DOPA decarboxylase activity in repeatedly pseudopregnant rats was less than the activity found in young diestrous (arcuate nucleus $p<0.05$, medial preoptic area $p=0.054$). I conclude that there are changes in DOPA decarboxylase activity during the estrous cycle and with aging.