EFFECT OF AGING ON TRYPTOPHAN HYDROXYLASE IN RAT BRAIN: IMPLICATIONS ON SEROTONIN LEVEL

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(Received March 9, 2000; accepted May 11, 2000)

This paper is available online at http://www.dmd.org

ABSTRACT:
Tryptophan hydroxylase (TrpH) catalyzes a rate-limiting step in the biogenesis of serotonin. The main objective of this study is to investigate the effect of aging on the activity of TrpH in serotonin-energized brain regions such as midbrain, pons, and medulla. TrpH activity was monitored by incubating various concentrations of tryptophan in a fixed amount of brain homogenate from midbrain, pons, and medulla of 2-month (young), 12-month (mature), and 24-month (old) rats. The product 5-hydroxytryptophan was quantitated using a reversed phase HPLC equipped with an electrochemical detection system. Michaelis-Menten constants, \( K_m \) and \( V_{max} \), were calculated using the Lineweaver-Burk plot. The affinity (\( K_m \)) of the enzyme significantly declined in midbrain and pons of old rats (141.1 ± 2.6, 126.0 ± 10.8 \( \mu M \)) relative to mature rats (22.4 ± 7.7, 38.2 ± 4.7 \( \mu M \)). However, no change was observed in medulla of old rats. The \( V_{max} \) of TrpH in pons of all three age group rats was fairly constant. However, the \( V_{max} \) of midbrain was significantly elevated, whereas that of medulla was reduced in old rats relative to mature rats. Clearance formation, a ratio of \( V_{max} \) to \( K_m \), of 5-hydroxytryptophan declined significantly in midbrain, pons, and medulla of old rats relative to mature rats. A combined effect of inefficient phosphorylation and oxidative damage of TrpH enzyme may be responsible for lower TrpH activity in aging brain. Such alterations in TrpH activity may reduce the level of serotonin in brain, which may be linked to late-life depression and other brain disorders, such as Alzheimer and Parkinson diseases.

A variety of human physiological functions have been shown to decline with aging (Shock, 1957). This decline in physiological functions may be associated with malfunctioning of the various autonomic systems in the body. One such system is the central nervous system in which aging causes a diminished function accompanied by changes in various neurotransmitter levels. Such variations in neurotransmitter levels may lead to various behavioral changes. It has been reported that aging causes a decrease in the concentration of various neurotransmitters in the brain (Meek et al., 1977; Sparks et al., 1985). Alterations in the brain concentration of serotonin can produce behavior abnormalities such as aggression, insomnia, suicidal or criminal behavior, loss of sex drive, despair, and/or misery often observed in elderly individuals (Frazer and Hensler, 1994). Reduction of serotonin level in aging may serve as a susceptibility factor in the development of late-life depression (Lerer et al., 1995). The prevalence of major depression is estimated at 1% to 10% of population aged 60 years or older, whereas depression may persist in up to 20% of the elderly population. Furthermore, the suicidal rate in this age group is higher than at any other stage of life (Casey, 1994). One possible reason for decreased serotonin level could be the decreased transport of precursor amino acid (tryptophan) across the blood-brain barrier.

Another possibility may be the diminished activity of the anabolic enzymes and/or enhanced activity of catabolic enzymes, which could lower the neurotransmitters level in aging brain. Transport of tryptophan, an amino acid precursor for serotonin, was found to decline in old rats (Tang and Melethil, 1995). A strong correlation between age-related mental disorders and brain neurotransmitters concentrations exists according to several studies that have reported reduced brain neurotransmitter levels associated with aging (Brizee, 1975; Sanatiago et al., 1988). Very little mechanistic information is available regarding age-related changes in brain serotonin levels. In this article we have described the effect of aging on tryptophan hydroxylase (TrpH)\(^{1}\), activity, a rate-limiting step involved in the biogenesis of serotonin in rat brain. The enzyme is localized in serotonergic neurons, which convert tryptophan to 5-hydroxytryptophan (5-HTP). TrpH is highly localized in midbrain, pons, and medulla (Frazer and Hensler, 1994), and we studied its kinetics in three different age group Fisher 344 BNFI rats, i.e., aged 2 months (young), 12 months (mature), and 24 months (old).

Experimental Procedures

**Chemicals.** Tryptophan, 5-HTP, dithiothreitol, \( \alpha \)-,\( \varepsilon \)-methyl-5,6,7,8-tetramethyl-ethyl hydroperoxide, and catalase were all obtained from Sigma Chemicals (St Louis, MO). Citric acid, EDTA disodium, and HPLC grade methanol were obtained from Fisher Scientific (St Louis, MO). Fisher 344 BNFI rats were obtained from Harlan NIA (Indianapolis, IN).

**Methods.** TrpH assay was adapted from a published procedure with minor modification (Sugden et al., 1989). Briefly, male Fisher 344BNFI rats were

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\(^{1}\) Abbreviations used are: TrpH, tryptophan hydroxylase; CI\(_e\), clearance formation; 5-HTP, 5-hydroxy tryptophan.

Support by a grant from Hoechst-Marion-Roussell. Support by the National Institute of Aging in providing animals of various age groups is gratefully acknowledged.

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1038
decapitated and midbrain, pons, and medulla were surgically isolated. Pooled brain regions were then homogenized in 5 volumes of 0.05 M Tris-HCl, pH 7.4, containing 2 mM dithiothreitol. The homogenate was centrifuged at 40,000g at 4°C for 30 min. Aliquots of supernatant was then preincubated with 1 mM NSD 1015 (decarboxylase inhibitor), 0.5 mM DL-6-methyl-5,6,7,8-tetrahydopterine (cofactor), and 1500 units of catalase at 37°C for 15 min. After preincubation, tryptophan (10–300 μM) was added and the mixture was incubated at 37°C for 45 min. The reaction was terminated by 1 N perchloric acid. Precipitated proteins were removed by centrifugation at 15,000g for 10 min. The metabolite (5-HTP) formed was then analyzed by HPLC using electrochemical detection. Protein estimation was done using the Lowry method (Lowry et al., 1951).

**HPLC Conditions.** The chromatographic separation was achieved by a reversed phase octadecyl silane column (Microsorb, particle size 5 μm; 150 × 4.6 mm i.d.). A short precolumn (60 × 5 mm i.d.) packed with octadecyl silane was used as a guard column. A mobile phase containing (0.05 M citric acid, 0.05 M sodium acetate, and 0.13 mM EDTA disodium (pH 6.0) with 5% v/v methanol as the organic modifier was employed. The flow rate was adjusted to 0.5 ml/min. Metabolite 5-HTP was detected using an electrochemical detector (Bioanalytical Systems, West Lafayette, IN). Glassy carbon was used as a working electrode. The working potential was set to +0.5 V with Ag/AgCl as the reference electrode. Using these chromatographic conditions, a clear separation of 5-HTP from other endogenous compounds was obtained. Tryptophan could not be detected at this potential, because it is oxidized at +0.9 V.

**Results**

The enzyme kinetic parameters of TrpH were obtained using the Lineweaver-Burk plot. Kinetic constants $K_m$ and $V_{max}$ of three brain regions, the midbrain, pons, and medulla, were calculated for young, mature, and old rats from Figs. 1, 2, and 3, respectively.

A variable trend with the age-related changes in the affinity of TrpH was observed in all three brain regions. In midbrain the affinity of the enzyme remained fairly unchanged in young to mature rats and then declined in old rats ($P < .01$) by almost 7-fold (Table 1). A biphasic phenomenon was also observed in pons. The affinity of TrpH was significantly elevated from young to mature rats ($P < .05$) and then declined significantly ($P < .05$) in old rats (Table 1). In medulla the affinity of the enzyme increased significantly from young to mature rats ($P < .05$) and then remained unchanged in the old animals. In all three age groups studied, the affinity of the enzyme in midbrain was significantly more than in pons and medulla except in old rats, where the affinity of the enzyme decreased drastically and leveled off in the pons and medulla.

An interesting phenomenon was observed in the midbrain of old rats. Contrary to our expectations, a 2-fold increase ($P < .01$) in the $V_{max}$ of old rats was noted relative to young and mature rats (Table 2). No statistical significant difference was observed in midbrain of young and mature rats. Pons was found to be the only region studied, where aging did not alter $V_{max}$. No significant changes ($P > .05$) were observed in the $V_{max}$ values of pons among rats of various age groups. A biphasic phenomenon was observed in medulla with a significant $V_{max}$ increase ($P < .05$) from young to mature rats followed by a significant decline ($P < .05$) in old rats (Table 2).

Clearance formation ($C_{I_{p}}$) of 5-HTP was calculated from the ratio of $V_{max}$ to $K_m$. No significant change in $C_{I_{p}}$ was observed in midbrain and pons of young rats relative to mature rats (Table 3). However, in the same brain regions almost a 3-fold decline in the $C_{I_{p}}$ of 5-HTP was observed in old rats in comparison to mature rats. A biphasic phenomenon was observed in medulla with an initial rise in $C_{I_{p}}$ from young to mature rats ($P < .05$) and then significant decline in old rats ($P < .01$). The magnitude of decline in $C_{I_{p}}$ of 5-HTP from mature to old rats in all three brain regions was approximately 3-fold.

**Discussion**

TrpH is the initial and rate-limiting enzyme involved in the biogenesis of the neurotransmitter serotonin (Jequier et al., 1967). It is also involved in the biogenesis of melatonin in the pineal gland. The present study provides evidence that TrpH activity is affected during the aging process, leading to a decreased level of serotonin in old rats (Meek et al., 1977; Sparks et al., 1985). TrpH from different regions of brain have different biochemical properties, suggesting that there are several isoforms of the enzyme. The basis of these biochemical
Reciprocal of corresponding tryptophan concentrations. $K_m$ and $V_{\text{max}}$ values are calculated from the $x$ and $y$ intercepts, respectively.

Reciprocal values of mean velocity ± S.D. of medulla are plotted against the reciprocal of corresponding tryptophan concentrations. $K_m$ and $V_{\text{max}}$ values are calculated from the $x$ and $y$ intercepts, respectively.

Differences remain unclear. A recent report suggested that TrpH mRNA levels are much higher in the rat pineal gland than in the brain stem, despite higher activity in the latter. This observation suggests that different forms of TrpH exist in the two tissues (Dumas et al., 1989). However, cDNA has been characterized from both tissues and found to be identical (Kim et al., 1991). These results support the hypothesis that tissue-specific differences in the properties, including TrpH activity, result from different post-translational modifications rather than pretranslational ones.

Among the brain regions we have studied, affinity of TrpH in midbrain and pons was considerably affected in old rats. The affinity of the enzyme in old rats declined almost 7-fold in midbrain and 3-fold in pons, in relation to mature rats. However, TrpH has been found to be less active in pons and medulla of young rats. This initial increase in the affinity of the enzyme in pons and medulla of mature rats and subsequent decrease in old rats in midbrain and pons may be due to variations in phosphorylation of the enzyme. TrpH activity is modulated by protein kinase A (Johansen et al., 1996) and calcium/calmodulin-dependent protein kinase (Furukawa et al., 1993). Phosphorylation of the enzyme occurs on serine 58 of the TrpH. A recent report suggests that replacement of serine 58 with arginine by site-directed mutagenesis considerably decreases the activity of TrpH (Kuhn et al., 1997). Phosphorylation of the enzyme is initiated by the...
activation of adenylate cyclase to generate second messenger cAMP. This second messenger, once formed, stimulates protein kinase A, which in turn phosphorylates TrpH. Any alteration in the phosphorylation cascade can subsequently modulate enzyme activity. Studies have shown that the activity of adenylate cyclase enzyme is enhanced in aging animals from young to mature rats (Araki et al., 1995) and subsequently diminished in old rats (Nomura et al., 1984; Hoskins and Ho, 1986). This initial increase and subsequent decrease of adenylate cyclase activity can be translated into variations in efficiency of phosphokinase A. This is consistent with our observations in pons and medulla, where the affinity of TrpH increased from young to mature rats and then decreased in old rats. The basal level of cAMP is maintained by its degradation with phosphodiesterases (3’5’-cAMP: nucleotide hydrolase). A significant increase in the high-K_m cAMP phosphodiesterase activity in the various brain regions has been reported in old rats relative to mature rats (Stancheva and Alova, 1991). This increase in the phosphodiesterase activity coupled with a decrease in the adenylate cyclase activity may alter the phosphorylation cascade of TrpH, leading to loss of TrpH activity with aging. TrpH is also phosphorylated by calcium/calmodulin-dependent protein kinase at serine 260 and serine 443 residues (Darmon et al., 1988). Several studies have reported that the levels of calmodulin, a calcium-binding protein, diminishes in various brain regions of old rats in comparison with young and mature rats (Teolato et al., 1983; Hoskins and Ho., 1986). Decreased levels of calmodulin in old rats can also indirectly affect the phosphorylation of TrpH by calcium/calmodulin-dependent protein kinase.

TrpH has been shown to be highly homologous with tyrosine hydroxylase (Grenett et al., 1987). A recent study has revealed that tyrosine hydroxylase in substantia nigra is inactivated by oxidation in old rats (De la Cruz et al., 1996). A similar effect may also be expected to cause a decline in TrpH activity along with inefficient phosphorylation in old rats. TrpH when incubated with catalase, which degrades H₂O₂, caused a significant elevation in the TrpH phosphorylation in old rats. TrpH when incubated with catalase, tyrosine hydroxylase in substantia nigra is inactivated by oxidation in calmodulin-dependent protein kinase. Hoskins and Ho., 1986). Decreased levels of calmodulin in old rats in comparison with young and mature rats (Teolato et al., 1983; Hoskins and Ho., 1986). Decreased levels of calmodulin in old rats can also indirectly affect the phosphorylation of TrpH by calcium/calmodulin-dependent protein kinase.

In conclusion, TrpH is significantly affected by aging in all three brain regions, i.e., midbrain, pons, and medulla, which can drastically effect the levels of serotonin in brain. A combined effect of inefficient phosphorylation and oxidative damage of TrpH is suggested as the probable cause of diminished activity in old rats. Further experiments are warranted to support this hypothesis.

References


