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

AZHER M. HUSSAIN AND ASHIM K. MITRA

Division of Pharmaceutical Sciences, School of Pharmacy, University of Missouri, Kansas City, Missouri

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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-enriched 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 (pH 7.4, 37°C). 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 μM) relative to mature rats (22.4 ± 7.7, 38.2 ± 4.7 μM). 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 (Scheme 1). 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) 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 BNF1 rats, i.e., aged 2 months (young), 12 months (mature), and 24 months (old).

Experimental Procedures

Chemicals. Tryptophan, 5-HTP, dithiothreitol, dl-6-methyl-5,6,7,8-tetramethyl hydroperidine, 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 BNF1 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 344BNF1 rats were supported by a grant from Hoechst-Marrion-Roussell. Support by the National Institute of Aging in providing animals of various age groups is gratefully acknowledged.
Results

The enzyme kinetic parameters of TrpH were obtained using the Lineweaver-Burk plot. Kinetic constants $K_{m}$ and $V_{\text{max}}$ 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. Midbrain in 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 $V_{\text{max}}$ 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 $V_{\text{max}}$. No significant changes ($P > .05$) were observed in the $V_{\text{max}}$ values of pons among rats of various age groups. A biphasic phenomenon was observed in medulla with a significant $V_{\text{max}}$ increase ($P < .05$) from young to mature rats followed by a significant decline ($P < .05$) in old rats (Table 2).

Clearance formation ($Cl_{f}$) of 5-HTP was calculated from the ratio of $V_{\text{max}}$ to $K_{m}$. No significant change in $Cl_{f}$ 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 $Cl_{f}$ 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 $Cl_{f}$ from young to mature rats ($P < .05$) and then significant decline in old rats ($P < .01$). The magnitude of decline in $Cl_{f}$ 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 values of mean velocity ± S.D. of pons are plotted against the reciprocal of corresponding tryptophan concentrations. $K_m$ and $V_{max}$ values are calculated from the x and y intercepts, respectively.

Among the brain regions we have studied, affinity of TrpH in midbrain and pons, and medulla of different age group (2, 12, and 24 month) rats from the Lineweaver-Burk plot as described under Experimental Procedures. Data were analyzed by ANOVA.

Statistical significance: * as compared with 2 months, $P < .01$; b as compared with 12 months, $P < .01$; c as compared with 12 months, $P < .05$; d as compared with 12 months, $P < .05$.

TABLE 1

<table>
<thead>
<tr>
<th>Regions</th>
<th>$K_m$</th>
<th>2 months</th>
<th>12 months</th>
<th>24 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu$mol/(\mu)mol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midbrain</td>
<td>30.5 ± 5.99</td>
<td>22.4 ± 7.7</td>
<td>141.1 ± 2.6$^{b, c}$</td>
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<tr>
<td>Pons</td>
<td>97.6 ± 12.92$^{c}$</td>
<td>38.2 ± 4.7</td>
<td>126.0 ± 10.8$^{d}$</td>
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<td>Medulla</td>
<td>124.3 ± 8.42$^{c}$</td>
<td>56.3 ± 0.1</td>
<td>95.6 ± 33</td>
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TABLE 2

<table>
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<th>2 months</th>
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<tr>
<td></td>
<td>$\mu$mol/min/mg protein</td>
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<td>Midbrain</td>
<td>0.253 ± 0.084</td>
<td>0.237 ± 0.014</td>
<td>0.567 ± 0.031$^{b, c}$</td>
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<td>Pons</td>
<td>0.273 ± 0.068</td>
<td>0.205 ± 0.013</td>
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<td>Medulla</td>
<td>0.156 ± 0.011$^{c}$</td>
<td>0.281 ± 0.011</td>
<td>0.199 ± 0.074$^{c}$</td>
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TABLE 3

<table>
<thead>
<tr>
<th>Regions</th>
<th>$Cl_f$</th>
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<th>12 months</th>
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<tr>
<td></td>
<td>$\text{mL/min}$</td>
<td></td>
<td></td>
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<tr>
<td>Midbrain</td>
<td>0.0082 ± 0.0012</td>
<td>0.0107 ± 0.0032</td>
<td>0.0040 ± 0.0001$^{c}$</td>
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<td>Pons</td>
<td>0.0028 ± 0.0004</td>
<td>0.0054 ± 0.0004</td>
<td>0.0014 ± 0.0001$^{b}$</td>
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<tr>
<td>Medulla</td>
<td>0.0012 ± 0.0001$^{c}$</td>
<td>0.0050 ± 0.0001</td>
<td>0.0020 ± 0.0001$^{c}$</td>
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Statistical significance: * as compared with 12 months, $P < .01$; b as compared with 12 months, $P < .01$; c as compared with 12 months, $P < .05$; d as compared with 12 months, $P < .05$.

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/calcmodulin-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 H_2O_2, caused a significant elevation in the TrpH activity, suggesting that the enzyme is prone to oxidation (Friedman et al., 1972). Furthermore, in a recent study TrpH has been shown to be susceptible to oxidative damage by reactive oxygen species (Cash, 1998). Studies are currently in progress in our laboratory to investigate the effect of oxidation on TrpH. We have also found that the activities of antioxidant enzyme, e.g., superoxide dismutase, glutathione peroxidase and catalase, undergo a significant age-related decrease in midbrain, pons, and medulla (AMH and AKM, unpublished results). A decrease such as this may lead to an increase in reactive oxygen species concentrations, which in turn may cause oxidative damage to TrpH.

No age-related changes were found in the V_max of TrpH in the pons, indicating a fairly constant activity of enzyme. However, an increase in the V_max of medulla from young to mature rats, which remains constant in old rats, suggests that the activity of TrpH increases in the prematurational phase. We found a 2-fold increase in V_max associated with the midbrain of old rats relative to mature rats. This may be due to a positive feedback mechanism, which is attempting to compensate for the 7-fold decrease in the affinity of the enzyme in old rats compared with mature rats. The other explanation could be a decline in the protein turnover rate by the proteolytic enzymes. Decrease in proteolytic enzyme susceptibility is known to occur due to formation of cross-linked aggregates of the denatured protein (Davies et al., 1987). A similar event can also be speculated in the midbrain of old rats. TrpH, being prone to oxidation as described earlier, can undergo aggregation and become less susceptible to protein turnover, subsequently causing increased V_max. Because the catalytic domain of the tryptophan has been shown to occur at the C terminus (Yang and Kaufman, 1991), aggregation by partial unfolding of the hydrophobic core might not result in total loss of activity. Furthermore, TrpH has been reported to exist in its catalytically active tetrameric form (Nakata and Fujisawa, 1982).

To comprehend the overall effect of the aging process on TrpH, Cl of 5-HTP formation can be calculated from kinetic parameters, V_max and K_m. The ratio of V_max to K_m generates the intrinsic clearence of tryptophan by TrpH. Tryptophan, being intrinsically cleared by the TrpH enzyme, may be considered as clearance formation of 5-HTP. In all three brain regions the Cl of 5-HTP declined in old rats in comparison with mature rats. This decline in Cl of 5-HTP can be translated into decreased levels of serotonin in aging animals, because the 5-HTP decarboxylation step is not the rate-limiting step. A 3-fold decrease in the Cl of 5-HTP in midbrain, pons, and medulla in old rats was revealed. This decline in the formation of 5-HTP may be adequate to affect the basal levels of serotonin, which in turn can be a major cause for late-life depression and other brain disorders. Although a clear relationship between the serotonergic system and Alzheimer disease has not yet been established, there is considerable evidence of a decreased brain level of serotonin in patients with Alzheimer disease (Reinikainen et al., 1990).

In conclusion, TrpH is significantly affected by aging in all three brain regions, i.e., midbrain, pons, and medulla, which can drastically affect 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


