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

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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 serotoninergic-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 quantified 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 between age-related mental disorders and brain neurotransmitters 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 behavioral 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, $\alpha$-6-methyl-5,6,7,8-tetramethyl hydroyperidine, 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

Abbreviations used are: TrpH, tryptophan hydroxylase; Cl, clearance formation; 5-HTP, 5-hydroxy tryptophan.
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 in all three brain regions almost a 3-fold decline in the old rats in comparison to mature rats. A biphasic phenomenon was observed in midbrain in old rats in comparison to mature rats. A biphasic phenomenon 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 \( V_{max} \) 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 the \( Cl_f \) 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 $V_{\text{max}}$ values are calculated from the $x$ and $y$ intercepts, respectively.

TABLE 1
Effect of age on the $K_m$ of tryptophan hydroxylase in different brain regions

<table>
<thead>
<tr>
<th>Regions</th>
<th>2 months</th>
<th>12 months</th>
<th>24 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midbrain</td>
<td>$30.5 \pm 5.99$</td>
<td>$22.4 \pm 7.7$</td>
<td>$141.1 \pm 2.6^{a,b}$</td>
</tr>
<tr>
<td>Pons</td>
<td>$97.6 \pm 12.9^{e}$</td>
<td>$38.2 \pm 4.7$</td>
<td>$126.0 \pm 10.8^{d}$</td>
</tr>
<tr>
<td>Medulla</td>
<td>$124.3 \pm 8.4^{e}$</td>
<td>$56.3 \pm 0.1$</td>
<td>$95.6 \pm 33$</td>
</tr>
</tbody>
</table>

Statistical significance: $^a$ 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, $P < .01$; $^e$ as compared with 12 months, $P < .05$.

TABLE 2
Effect of age on the $V_{\text{max}}$ of tryptophan hydroxylase in different brain regions

<table>
<thead>
<tr>
<th>Regions</th>
<th>2 months</th>
<th>12 months</th>
<th>24 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midbrain</td>
<td>$0.253 \pm 0.084$</td>
<td>$0.237 \pm 0.014$</td>
<td>$0.567 \pm 0.031^{a,b}$</td>
</tr>
<tr>
<td>Pons</td>
<td>$0.273 \pm 0.068$</td>
<td>$0.205 \pm 0.013$</td>
<td>$0.182 \pm 0.020$</td>
</tr>
<tr>
<td>Medulla</td>
<td>$0.156 \pm 0.01^{d}$</td>
<td>$0.281 \pm 0.011$</td>
<td>$0.199 \pm 0.07^{d}$</td>
</tr>
</tbody>
</table>

Statistical significance: $^a$ 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, $P < .01$; $^e$ as compared with 12 months, $P < .05$.

TABLE 3
Effect of age on the $C_l$ of 5-hydroxytryptophan in different brain regions

<table>
<thead>
<tr>
<th>Regions</th>
<th>2 months</th>
<th>12 months</th>
<th>24 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midbrain</td>
<td>$0.0082 \pm 0.0012$</td>
<td>$0.0107 \pm 0.0032$</td>
<td>$0.0040 \pm 0.0001^{a}$</td>
</tr>
<tr>
<td>Pons</td>
<td>$0.0028 \pm 0.0004$</td>
<td>$0.0054 \pm 0.0004$</td>
<td>$0.0014 \pm 0.0001^{b}$</td>
</tr>
<tr>
<td>Medulla</td>
<td>$0.0012 \pm 0.0001^{c}$</td>
<td>$0.0050 \pm 0.0001$</td>
<td>$0.0020 \pm 0.0001^{c}$</td>
</tr>
</tbody>
</table>

Statistical significance: $^a$ as compared with 12 months, $P < .01$; $^b$ as compared with 12 months, $P < .05$; $^c$ as compared with 12 months, $P < .01$; $^d$ 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/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 (Teoletal., 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 tyrosine hydroxylase in substantia nigra is inactivated by oxidation in old rats (Grenett et al., 1987). A recent study has revealed that the activity of tyrosine hydroxylase is reduced in substantia nigra by cross-linked aggregates of the denatured protein (Davies et al., 1987). This increase in the phosphodiesterase activity coupled with a decrease in the 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 (Teoletal., 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 affect the basal 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


