Exercise Training Reverses the Age-Related Decline in Tyrosine Hydroxylase Expression in Rat Hypothalamus

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Tyrosine hydroxylase (TH) is the rate-limiting enzymatic step in the catecholamine biosynthesis pathway. Some studies have demonstrated that aging is associated with a decrease in TH activity and TH mRNA in rat hypothalamus. We previously demonstrated that exercise training can decrease TH gene expression in the adrenal medulla of young but not senescent rats. This study was designed to examine the effects of endurance training on the TH expression in hypothalamus with aging. To this end, we assessed TH mRNA, TH immunoreactivity, and TH activity with or without exercise training. Young and old F-344 female rats were trained by treadmill running for 8 weeks. All parameters examined were significantly lower in hypothalamus of old (25-month) compared with young (5-month) control animals (p < .05). Exercise training significantly elevated TH mRNA (n = 5-7 in each group), TH immunoreactivity (n = 5-8 in each group), and TH activity (n = 12-13 young groups and n = 6 old groups) in the hypothalamus of old animals (p < .05), but there was no significant change in any of these parameters in young animals following training. These data indicate that endurance training can reverse the age-related decline in catecholamine biosynthesis in the hypothalamus.

Tyrosine hydroxylase (TH), the rate-limiting enzymatic step in the catecholamine biosynthesis pathway (Nagatsu et al., 1964) plays a central role in the neuronal transmission and hormonal action of catecholamines. Hypothalamic production of tyrosine-derived neurotransmitters may be important for the subsequent secretion of other peptide hormones, such as gonadotrophic, thyroid, and growth hormones (Meites, 1991). Although some investigators report no changes with age, many reports show that resting circulating catecholamines are elevated with age in both humans and animals (Ziegler et al., 1976; Roberts and Tümer, 1987; Kohrt et al., 1993). This could be due to the increased adrenomedullary activity with senescence (Strong et al., 1990; Tümer et al., 1992; Tümer and LaRochelle, 1995). The sympathetic effenter nerves innervating the adrenal medulla have increased neural activity with age, and this increase in neural activity is paralleled by increased catecholamine secretion from the medulla (Ito et al., 1986).

Alterations in the expression of TH with aging are tissue-specific in rodents. Whereas TH activity, TH mRNA, and TH immunoreactivity levels are increased with age in adrenal medulla (Reis et al., 1977; Kedzierski and Porter, 1990; Strong et al., 1990; Voogt et al., 1990; Tümer et al., 1992; Tümer and LaRochelle, 1995), TH mRNA levels do not change in superior cervical ganglion (Reis et al., 1977; Kedzierski and Porter, 1990). In the brain, the changes with age are more controversial. For example, TH mRNA and TH activity have been reported to be either increased (Reis et al., 1977; Harik and McCracken, 1986), decreased (McGeer et al., 1971; Ziegler et al., 1976; Reymond et al., 1984; Voogt et al., 1990; Zoli et al., 1993; Himi et al., 1995), or unchanged (Algeri et al., 1982; Kedzierski and Porter, 1990). Specifically, one report demonstrated increased TH activity in the hypothalamus (Reis et al., 1977), and another reported decreased TH activity (Reymond et al., 1984). Several reports showed no change in TH activity or TH mRNA (Algeri et al., 1982; Toffano et al., 1982; Kedzierski and Porter, 1990), whereas one found decreased TH mRNA (Voogt et al., 1990).

Turnover and circulating levels of peripheral catecholamines, measured both at rest and in response to exercise, are markedly reduced by exercise training in rodents (Ostman et al., 1972; Ostman and Nyback, 1976; Mazzeo et al., 1986). We previously demonstrated that exercise training can decrease TH gene expression in the adrenal medulla of young but not senescent rats (Tümer et al., 1992).

To determine if exercise training modulates catecholamine biosynthesis in the hypothalamus in young or senescent rats, we assessed TH mRNA, TH immunoreactivity, and TH activity in the hypothalamus of female Fischer-344 (F-344) rats of 5 and 25 months of age with or without exercise training.

METHODS

Animals. — F-344 female rats 3 and 23 months old were obtained from a colony maintained for the National Institute on Aging at Harlan-Sprague Dawley Laboratories (Indianapolis, IN). On arrival, rats were housed individually under barrier conditions in a temperature-regulated environment (26 ± 1 °C) on a 12-hour light/dark cycle. No serologic or bacteriologic tests were performed. At the
time of sacrifice, rats were either 5 or 25 months of age. Maximum life span for F-344 rats maintained under these conditions is approximately 35 months, with a mortality rate of about 50% at 29 months (Coleman et al., 1977).

Exercise training. — Rats were adjusted to a reverse day:night cycle with lights on at 6 p.m. and lights off at 6 a.m. Food and water were available ad libitum. The rats were randomly assigned to either the control group or the exercise training group. The rats were exercised during the dark period by treadmill running, as previously published (Scarpace et al., 1994) at approximately 70% of their peak oxygen consumption (peak VO₂, see next section) 5 days/week over 9 weeks. Each training session began with a 5-min warm-up at ~15 m/min (0% grade). On day 1 (week 1) of training, the animals began exercising at 25 m/min (young) or 18 m/min (old) with a 10-min duration at 0% grade. The duration of exercise was increased by 2 min/day until the animals reached 60 min of exercise. The exercise time period remained at 60 min for the final 4 weeks of the study. During weeks 1–3, the grade was gradually increased from 0% to 5%. Beginning at week 4, the speed was increased to 30 m/min (young) and 20 m/min (old) and the grade reset to 0%. During weeks 4–9, the grade was gradually increased to 12.5%. At the end of the ninth week, the rats were exercising for 60 min at a 12.5% grade at speeds of 30 m/min (young) and 20 m/min (old). Because the VO₂ max was less in the senescent rats (see next section), the final training speed for running was less in these animals. Animals were continuously monitored during exercise to verify that running was maintained. Nonexercised animals were placed into the treadmill for equal lengths of time with the apparatus in the "off" position. Rats were sacrificed 48 hr after the last exercise session. All rats in the exercise groups ran with minimal electrical stimulation. In the exercise groups, 2 young rats out of 15 and 5 old rats out of 13 did not complete the studies because of injuries (2 young, 2 old) or death unrelated to running (3 old). In the control groups, 2 old rats out of 12 died. All 12 young control rats completed the study. Complete assessment (TH mRNA, TH immunoreactivity, and TH activity) was not accomplished for every rat.

Measurement of peak VO₂. — Peak VO₂ was determined prior to initiation of training and during the last week prior to sacrifice. Peak VO₂ was measured on 3 to 5 animals in each experimental group by using a flow-through open-circuit system. Briefly, the animals performed an incremental exercise protocol that began with a 4-min warm-up followed by a work rate increase every 4 min until the animals were unable to maintain the required running speed. The protocol was designed such that peak VO₂ was reached within 8–12 min after the warm-up. Room air was pulled through the treadmill chamber (Omnitech Electronics, Columbus, OH) at a flow rate of 4.5 L/min. Gas was sampled (500 ml/min) from a small mixing chamber located at the top of the treadmill and analyzed for O₂ content by using electronic gas analyzers. The gas analyzers were calibrated immediately before and after each experiment with standardized gases. O₂ uptake was calculated by using the Haldane transformation of the Fick equation (Powers et al., 1987).

Tissue preparation. — Animals were anesthetized with pentobarbital, and the brains were removed quickly and immediately frozen on dry ice. Tissues were stored at -80 °C. At the time of assay, brains were dissected and hypothalamus weighed and homogenized in 100 μl of phosphate buffer (2 mM NaPO₄, 0.2% Triton, pH 7.0). Protein was determined by the method of Bradford (1976).

TH activity. — TH activity was measured using a radioenzymatic assay as described previously (Tümer and LaRochelle, 1995) and based on a modification of the assay by Reinhard et al. (1986). Briefly, 25 μl of homogenate were analyzed at pH 7.0 in the presence of 6-MPH₄ (1.5 mM) and [3,5-³H]tyrosine (100 μM; 1 μCi/reaction), in a total volume of 50 μl for 15 minutes at 37 °C. The assay is based upon the release of ³H₂O from [³H]-[3,5]-L-tyrosine with absorption of the isotopic substrate (and its metabolites) by an aqueous slurry of activated charcoal. Unbound ³H₂O was analyzed by liquid scintillation spectrometry.

TH mRNA. — TH mRNA was determined in the hypothalamus using our previously published method (Tümer et al., 1992), with a modification. Briefly, sonicated tissue (75 μl homogenate) was extracted with RNAzolB (a mixture of phenol and guanidinium thiocyanate; Biotec, Friendswood, TX; Chomczynski and Sacchi, 1987). The integrity of the isolated RNA was verified using agarose (1%) gel electrophoresis in comparison with 18S and 28S RNA standards (Sigma, St. Louis, MO). The pBR322 recombinant plasmid containing the TH.36cDNA probe (Brown et al., 1987), kindly supplied by Dr. Karen O'Malley (Washington University, School of Medicine), was grown in E. coli, and plasmid DNA was isolated by standard procedures (Tümer and LaRochelle, 1995). Several concentrations of serially diluted RNA samples were immobilized on nylon membranes (Gene Screen, New England Nuclear, Boston, MA) using a Bio-Rad (Richmond, CA) slot blot apparatus. After prehybridization, filters were hybridized with a ³p random primer-generated rat TH.36cDNA probe to bind the sample THmRNA. After hybridization, the filters were washed and exposed to phospho screen for 72 hours using PhosphoImager (Molecular Dynamics, Sunnyville, CA). The screens were scanned, and volumes for each sample were calculated from the counts per pixel using Image Quant software (Molecular Dynamics). The images (volume) were normalized by comparison with internal laboratory standards of rat adrenal medullary RNA present on each nylon membrane. Experimental values were within the linear range of the standards.

TH immunoreactivity. — TH protein levels were determined using our previously described methods (Tümer and LaRochelle, 1995). Briefly, tissue homogenates were diluted in phosphate buffer containing 1% SDS and boiled for 10 min. Samples were then dot-blotted onto nitrocellulose membranes (Bio-Rad). Immunoreactive protein was ass-
sessed by using polyclonal antibody to TH IgG (Pel-Freez Biologicals, Rogers, AR) and HRP-labeled donkey anti-rabbit IgG (Amersham Life Sciences, Arlington Heights, IL) and visualized by using chemiluminescent detection (Amersham), quantified by video densitometry (Bio-Rad).

**Statistical analysis.** — Comparisons among ages and with training were determined by two-way analysis of variance (ANOVA) and Fisher's protected LSD post hoc test. Analyses were performed using super ANOVA (Abacus Concepts, Berkeley, CA).

**RESULTS**

The body weights of the young rats, as expected, were considerably less than the senescent rats (193 ± 3 g vs 270 ± 4 g; p < .03). Training had no significant effect on body weight in rats of either age (191 ± 3 g young; 262 ± 5 g old; p > .05). After 9 weeks of training, the maximum oxygen consumption (while running) significantly increased by 12% and 10%, respectively, for young (86.8 ± 2.8 ml/kg/min vs 97.7 ± 1.9, p < .005) and old (69.7 ± 2.6, vs 76.4 ± 2.0, p < .05) rats, compared to pretraining levels as previously reported (Sullivan et al., 1995). This indicated that a similar level of training occurred in both ages of rats.

To determine the effect of exercise training on catecholamine biosynthesis, TH activity was determined in the hypothalamus of young and old rats with and without exercise training. In sedentary rats, TH activity was significantly lower in the hypothalamus of 25-month-old compared with the 5-month-old rats (Table 1). To determine if this decrease with age was due to a different requirement for the cofactor 6 MPH, pterin dose response curves were assessed (Figure 1). Maximum enzyme activity was achieved at 2 mM pterin concentrations in both age groups. Above this concentration there was inhibition of TH activity. These data indicated that the diminution in hypothalamus TH activity with age was not due to a different requirement for the cofactor.

There was a significant effect of exercise on TH activity (Table 1). In addition, there was a significant interaction between age and exercise, indicating that exercise training had different effects on TH activity in young and old rats. In young rats, there was no significant change in TH activity. In contrast, training significantly elevated TH activity in the hypothalamus of senescent rats to the extent that there was no longer a difference with age (Table 1).

To determine if the effects of training on TH activity were due to changes in the amount of TH protein, TH immunoreactivity was assessed in homogenates of hypothalamus following training in young and old rats. Similar to the decrease in TH activity, TH immunoreactivity was significantly less in old control rats compared with young controls. Endurance training had no significant effects on TH immunoreactivity in young rats. In contrast, TH immunoreactivity was significantly increased in 25-month-old animals following training (Table 2).

The effect of endurance training on TH mRNA was also assessed to determine if the alterations in TH protein are a consequence of gene expression. Northern analysis indicated that the employed probe hybridizes to a single mRNA species corresponding to 2.1 kb (data not shown), which is similar to what has been previously reported for the rat (Lewis et al., 1983). Table 3 represents the results of hypothalamus TH mRNA quantification by slot blot analysis. Similar to the findings with TH activity and immunoreactivity, there was a significant age-related decline in TH mRNA levels of old rats compared with young controls. Although endurance training had no significant effect on TH mRNA in the hypothalamus of 5-month-old animals, TH mRNA was significantly elevated in 25-month-old animals as compared to the control group (Table 3).
Tyrosine-derived neurotransmitters, including dopamine and norepinephrine synthesized within the hypothalamus, are important modulators of hypophysiotropic peptide hormones. In rats there is evidence that norepinephrine and dopamine synthesis decline with age. This may be important in the decreased gonadotrophic hormone secretion, cessation of estrous cycles, reduced testosterone secretion, diminished thyroid hormone secretion, and the decline in growth hormone and IGF-1 secretion with age (Meites, 1991). The decrease in catecholamine synthesis in the hypothalamus with age (Ponzio et al., 1978) may be a result of diminished TH activity. The present study demonstrated a 22–24% decrease in TH activity and TH immunoreactivity and a greater than 50% decrease in TH mRNA. The decrease in TH activity was similar to that reported by Raymond et al. (1984) in Long Evans rats, but in contrast to that reported by others, indicating either no change or an increase with age (Reis et al., 1977; Algeri et al., 1982; Ponzio et al., 1982; Kedzierski et al., 1990). Each of the aforementioned studies used either a different rat strain or gender. This may explain the discrepancies among the findings. In humans, there are far fewer studies, but there is evidence that TH activity declines in the hypothalamus with age (Meites, 1991).

The age-related differences in TH activity, TH immunoreactivity, or TH mRNA in other regions of the brain as well as in the peripheral nervous system are also not consistent in the published literature. They have been reported to be either increased (Reis et al., 1977; Harik and McCracken, 1986), decreased (McGeer et al., 1971; Ziegler et al., 1976; Reymond et al., 1984; Himi et al., 1995) or unchanged (Algeri et al., 1982; Kedzierski and Porter, 1990). Moreover, our studies using the female F-344 rat suggest that the regulation of TH biosynthesis is tissue-specific. In the same genetic strain of animals used in the present study, where we found a decrease in TH activity in hypothalamus with age, we previously reported that TH activity, TH immunoreactivity, and TH mRNA increased with age in the adrenal medulla (Tümér and LaRochelle, 1995).

There have been numerous attempts to reverse the consequences of diminished brain catecholamines with age, and some of these have met with success. Treatment with drugs to raise brain catecholamines significantly prolonged life span in both mice and rats, improved sexual function in rats, and improved memory retention in mice (Meites, 1991). Exercise is generally considered to be beneficial (McHenry et al., 1990) and is known to both increase and decrease catecholamine syntheses including the modulation of TH activity. We have previously reported that exercise training reduces TH expression in adrenals of young rats, but there was no significant change in the old rats (Tümér et al., 1992). In the present study, the response to training was also different in the senescent rats, but opposite of what we found in the adrenals. The senescent rats upregulated catecholamine biosynthesis with exercise training, whereas the young rats did not. Exercise training had no significant effect on the level of TH activity, TH immunoreactivity, and TH mRNA in the hypothalamus of young rats. In contrast, training increased these same parameters in senescent rats, such that exercise training reversed the age-related decline in catecholamine biosynthesis.

The mechanism of exercise-induced modulation of TH gene expression is unknown. We have previously shown that GABAergic and cholinergic systems may play a role in exercise-induced alterations in TH gene expression in the adrenal gland (Tümér et al., 1992). This mechanism could

### Table 1. TH Activity in Hypothalamus With and Without Exercise Training in Young and Old Rats

<table>
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<tr>
<th></th>
<th>5-month-old Rats</th>
<th>25-month-old Rats</th>
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<tbody>
<tr>
<td>Control</td>
<td>3.3 ± 0.1</td>
<td>2.5 ± 0.6*</td>
</tr>
<tr>
<td>Trained</td>
<td>3.4 ± 0.2</td>
<td>3.5 ± 0.2*</td>
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Notes: Data represent mean ± SE of 12–13 (young) and 6 (old) rats. p < .01 for difference between trained and control and p < .05 for interaction between age and training by 2-way ANOVA.

* p < .005 for difference between young and old control rats by Fisher's Protected LSD.

* p < .02 for difference between trained and control in old rats by Fisher's Protected LSD. Other groups were not significantly different from each other.

### Table 2. Effects of Age and Exercise Training on Hypothalamus TH Immunoreactivity in Rats

<table>
<thead>
<tr>
<th></th>
<th>5-month-old Rats</th>
<th>25-month-old Rats</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>6.8 ± 0.3</td>
<td>5.3 ± 0.1*</td>
</tr>
<tr>
<td>Trained</td>
<td>6.5 ± 0.1</td>
<td>7.4 ± 0.9*</td>
</tr>
</tbody>
</table>

Notes: Data represent mean ± SE of 5–7 rats in each age group. p < .001 for difference between trained and control and p < .001 for interaction between age and training.

* p < .001 for difference between young and old control rats by Fisher's Protected LSD.

* p < .05 for difference between trained and control old rats by Fisher's Protected LSD. Other groups were not significantly different from each other.

### Table 3. Hypothalamus TH mRNA Following Exercise Training in Young and Old Rats

<table>
<thead>
<tr>
<th></th>
<th>5-month-old Rats</th>
<th>25-month-old Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>58.2 ± 4.3</td>
<td>23.3 ± 6.2*</td>
</tr>
<tr>
<td>Trained</td>
<td>70.7 ± 5.5</td>
<td>56.9 ± 3.7*</td>
</tr>
</tbody>
</table>

Notes: Data represent mean ± SE of 5–7 rats in each age group. p < .001 for difference between trained and control; p < .001 for difference between young and old; and p < .05 for interaction between age and training.

* p < .001 for difference between young and old control rats by Fisher's Protected LSD.

* p < .001 for difference between trained and control old rats by Fisher's Protected LSD. Other groups were not significantly different from each other.
be operating in the hypothalamus as well. Additional investigations are needed to examine the trans-synaptic regulation of TH indication in the hypothalamus following training. Nevertheless, our findings demonstrating that exercise training reverses the age-related decrease in catecholamine biosynthesis in the hypothalamus suggest that exercise may be beneficial in reversing some of the neuroendocrine consequences of impaired hypothalamic catecholamine synthesis with age.

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