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Lifetime Brain Serotonin: Regional Effects of Age and Precursor Availability

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TIMIRAS, P. S., D. B. HUDSON AND P. E. SEGALL. *Lifetime brain serotonin: Regional effects of age and precursor availability*. NEUROBIOL AGING 5(3) 235–242, 1984.—In the rat, regional brain serotonin levels which do not change from 2–30 months of age are increased at 36 months. Corresponding catecholamine levels progressively decrease. Feeding a diet restricted in the amino acid tryptophan (the precursor of serotonin) from weaning to two years of age markedly reduces serotonin levels in all brain regions and lowers norepinephrine levels in the cerebral hemispheres. Regional activity of synthesizing (tyrosine and tryptophan hydroxylases) and catabolizing enzymes (MAO-A) does not change markedly with age or dietary manipulation except for sporadic increases in tryosine hydroxylase activity in pair-fed animals. Returning the tryptophan-deficient animals to a normal diet produces a certain degree of rehabilitation the effectiveness of which varies with the function considered: Impaired brain serotonin levels recover moderately but remain lower than controls as late as 36 months, growth is never completely compensated, and norepinephrine levels show a rebound increase.

Brain serotonin changes Tryptophan-restriction Aging Brain monoamine imbalance with aging

WITH aging the levels and metabolism of cholinergic and catecholaminergic systems in discrete brain regions decline progressively while the incidence and severity of certain neurologic and mental disorders increase (e.g., loss of striatal dopamine in Parkinson's disease and loss of cortical acetylcholine in Alzheimer's disease and some types of senile dementia) [3, 11, 23]. In contrast, chemical and morphologic studies of the rat brain suggest that serotonin levels remain unchanged with normal aging [1, 8, 32–36]. Likewise, in humans, aging alone does not result in reduced brain serotonin levels and, in abnormal aging (e.g., Alzheimer's disease), serotonin receptors are decreased in younger but not in older patients in whom the prevalent loss is cholinergic [3,6]. While preweaning development of brain serotonin has been well described, little is known of its regional distribution and metabolism in maturity or with aging.

The primary purpose of this study was to obtain a normal profile of brain serotonin throughout the lifespan. In our experiments, brain serotonin as well as norepinephrine and dopamine were measured in rats at progressive ages: 2 months, (upon achievement of sexual maturity), 4, 6, and 12 months (during adulthood) and 24, 30 and 36 months (progressively older ages). On the assumption that regional differences in the timetable of development may persist as well in aging, we compared regions with high densities of serotonergic cell bodies (e.g., pons-medulla) with those rich in serotonergic terminals (e.g., cerebral cortex), or areas involved with endocrine activity (e.g., hypothalamus).

A secondary purpose was to study the effects of long-term dietary tryptophan restriction in developing, adult and senescent rats. On the premise that some neurotransmitter levels in the brain may be modified by manipulation of their precursors in the diet [3, 5, 11, 12, 39], we wanted to investigate whether lowering the dietary intake of the serotonin precursor, tryptophan, early during development and throughout adulthood might later influence the timetable of aging of serotonergic and, indirectly, catecholaminergic systems. Low levels of tryptophan in the diet caused voluntary restriction of food intake and reduced body growth, therefore we included an additional group of animals (pair-fed) fed a qualitatively complete diet but in reduced amounts to match those consumed under tryptophan restriction.

METHOD

Animals

At 21 days of age 240 female Long-Evans rats were assigned to five groups: (1) control group fed Purina rat chow ad lib; (2) a group fed ad lib a specially formulated diet supplemented with an amount of tryptophan equivalent to the amount in Purina rat chow; (3 and 4) two groups (T30% and T40%) receiving ad lib the specially formulated diet containing progressively lower levels of tryptophan; and (5) a (pair-fed) group fed Purina rat chow equal in weight to the food consumed by groups 3 and 4 (Table 1). Comparison of the effects of diet in control (1) (Purina) and control (2) (Teklad diet with normal tryptophan levels) shows no differences within any of the parameters considered. Therefore, all values from both groups were added in calculating the mean and standard error for control values. Water was available ad lib to all groups. Temperature was maintained at 22°C±2° and lighting was on a 12/12 schedule.

At 2, 4, 6, 12 and 24 months of age five animals from each group were sacrificed. At 24 months of age most

TABLE 1

TRYPTOPHAN CONTENT OF DIETS AND AMOUNTS INGESTED DAILY BY ADULT FEMALE RATS*

	Diet Group Designation			
	T30%	T40%	Control† (T100%)	Pair-fed (T100%)
Tryptophan‡ Content	0.62 mg/g	0.81 mg/g	2.07 mg/g	2.07 mg/g
Daily food intake	5 g	5 g	15 g	5 g
Daily tryptophan intake	3.1 mg	4.05 mg	31.05 mg	10.35 mg

*All experimental diets were prepared according to our instructions by Teklad Test Diets, Madison, WI. They consisted: caseine hydrolysate acid (salt free) 150 g/kg; sucrose, 4 g/kg; corn oil, 50 g/kg; mineral mix, Jones Foster 50 g/kg; vitamin mix, Teklad, 10 g/kg. Tryptophan, an essential amino acid is particularly low in corn which was selected as a primary protein source. The progressive restoration of tryptophan was accomplished by an equivalent reduction in the amount of ground corn which varies from 722 g/kg in T30%, 721.85 g/kg in T40% and 720.5 g/kg in T100% and the addition of tryptophan: 0.0 g/kg in T30%; 0.15 g/kg in T40% and 1.5 g/kg in T100%. The tryptophan content of the T100% diet was the same as that of Purina Rat Chow (Ralston Purina Co., St. Louis, MO.)

†Controls were fed ad lib either Purina Rat Chow or T100% diet, the tryptophan content being equivalent. Pair-fed animals were fed Purina Chow in amounts similar to those consumed by the rats fed the tryptophan restricted diets.

‡Tryptophan content of all diets was assayed microbiologically according to the method of Wooley and Sebral, J. Biol. Chem. 157:141, 1945 by Raltech Scientific Services, Division Ralston Purina Co.

tryptophan-restricted rats were returned to the control diet; surviving control and refed rats were also sacrificed at 30 and 36 months. Pair-fed rats were not studied beyond 24 months. Females were used because of our interest in reproduction, results of this part of the work have been reported elsewhere [30]. Data on a restricted number of 30–36 month animals are included to indicate the trend at these later ages, not for statistical purposes.

Animals in all experiments were sacrificed by decapitation, the brain was immediately placed on ice, and rapidly divided into five areas: cerebral hemispheres (minus olfactory lobes and caudate nucleus), caudate nucleus, hypothalamus, mesodiencephalon and pons-medulla.

Neurochemical Analyses

The monoamines, serotonin, norepinephrine, and dopamine, were extracted and assayed by our modification of the method of Chang [9] utilizing half of each brain region, except the hypothalamus, where the entire tissue was used.

Extraction. Tissue (280 mg or less) was sonicated in 2.8 ml acidified n-butanol (0.85 ml concentrated HCl/L) and centrifuged at 900 g for 15 minutes. The supernatant (2.5 ml) was extracted with 5 ml heptane and 1.2 ml glass-distilled water by shaking for 20 minutes. After centrifugation at 300 g for 10 minutes, the upper organic phase and tissue interface were aspirated and the water phase aliquotted for the assays. Blanks and standards of 25–100 ng of each monoamine were extracted as above in similiar volumes. Sample concentrations, in nanograms, were determined by comparison to these standards and calculated per milligram wet tissue weight.

Serotonin. An aliquot (0.4 ml) of the water phase was reacted with 1.0 ml O-phthaldehyde (OPT, Regis Chemical Corp., 10 mg/100 ml 10 N HCl) and heated in water at 100°C for 10 minutes. After cooling, the resulting fluorescence was read at 355/470 nm on a Perkin-Elmer spectrofluorometer Model MPF-44A.

Norepinephrine-Dopamine. An aliquot (0.6 ml) of the water phase was added to 3 ml 2 N sodium acetate and 200 mg activated acid aluminum oxide (Acid Alumina, Bio-Rad), shaken for 20 minutes, centrifuged at 300 g for 10 minutes and the water phase aspirated. The alumina was washed two times with 5 ml water. The monoamines were eluted with 1.2 ml 0.1 M acetic acid. The eluate (1.0 ml) was adjusted to pH 6.5 with a sodium acetate-EDTA buffer and oxidized for exactly 2 minutes with an iodine-potassium iodide solution. The reaction was stopped with alkaline sulfite and the pH adjusted to 5.4 with glacial acetic acid. Samples were heated in water for 2 minutes at 100°C, cooled, and the norepinephrien fluorescence read at 385/485 nm. The samples were heated for an additional 10 minutes to develop the dopamine fluorescence which was read at 325/380 nm.

Enzyme Assays

Enzyme assays were performed in duplicate immediately after the supernatant preparation; optimum conditions for enzyme reactions had been previously assessed.

Tryptophan hydroxylase. Tryptophan hydroxylase was assayed by our modification of the method of Ichiyama *et al.* [16]. Tissues were assayed fresh, immediately after sacrifice. A 10% homogenate was prepared in 10 mM tris acetate pH 7.4 buffer containing 1 mM DTT and centrifuged at 50,000 g for 30 minutes. Duplicate 500 μl aliquots of the supernatant were incubated at 37°C for 30 minutes with a substrate solution at pH 7.4 containing 1.4 mM L-(l-^{14}C) tryptophan (58 mCi/nmole SpA., NEN) and 100 μM L-tryptophan (final concentrations). The reaction was stopped by the addition of 6 N PCA and the incubation, at 37°C, continued for 2 hours.

TABLE 2

REGIONAL MONOAMINE LEVELS IN ADULT AND AGED FEMALE RATS ON CONTROL DIETS*

Age	2 months	4 months	6 months	1 year	2 years	2.5 years	3 years
				Serotonin (ng/g)			
Cerebral Hemispheres	425 ± 16†	465 ± 33	528 ± 28	586 ± 37	522 ± 23	501 ± 15	742
Corpus Striatum	922 ± 47	1179 ± 60	1003 ± 39	1378 ± 118	1083 ± 60	1039 ± 99	1866
Hypothalamus	1994 ± 68	1898 ± 92	1861 ± 117	—‡	1830 ± 85	1934 ± 139	2669
Mesodiencephelon	1193 ± 79	1202 ± 45	1194 ± 53	1245 ± 31	1402 ± 77	1163 ± 92	1779
Pons Medulla	1081 ± 91	1051 ± 91	1103 ± 39	1303 ± 56	1170 ± 75	1025 ± 18	1618
				Norepinephrine (ng/g)			
Cerebral Hemispheres	229 ± 18	238 ± 16	273 ± 20	237 ± 20	242 ± 11	220 ± 24	181
Hypothalamus	2395 ± 107	1771 ± 96	1849 ± 130	—	1429 ± 111	1394 ± 113	1280
Mesodiencephelon	539 ± 37	440 ± 38	403 ± 45	533 ± 18	456 ± 18	508 ± 15	392
Pons Medulla	447 ± 38	466 ± 64	373 ± 16	521 ± 32	487 ± 18	563 ± 109	361
				Dopamine (ng/g)			
Cerebral Hemispheres	674 ± 41	837 ± 134	806 ± 98	421 ± 41	468 ± 23	599 ± 11	575
Corpus Striatum	10520 ± 414	13666 ± 871	12274 ± 954	9235 ± 633	8892 ± 359	10928 ± 1158	7984

*Diets (Purina and Teklad described in Table 1) contain normal amounts of tryptophan.
†Average ±SE for 10 animals per group, sacrificed 1000–1200 hr. Two animals for 3-year-old group.
‡Serotonin not measured in hypothalamus at one year of age.

The $^{14}CO_2$ evolved in the reaction was trapped by a folded filter paper embedded with 200 μl Hyamine (NEN) in a plastic center well. Center well was transferred to a scintillation vial containing 10 ml Dimiscint (National Diagnostics). The samples were counted for 10 minutes after dissipation of the initial chemiluminescence effect.

Tryosine hydroxylase. Tyrosine hydroxylase was assayed according to the method of Waymire *et al.* [37]. Duplicate 50 μl aliquots of a 10% homogenate in 0.32 M sucrose were added to a substrate at pH 7.4 containing a final concentration of 20 nM L-(l-^{14}C)-tyrosine (50 mCi/nmole SpA., NEN) and 80 nM L-tyrosine. Samples were incubated at 37°C for 20 minutes in a shaker bath. The reaction was stopped by the addition of 10% TCA and the incubation at 37°C was continued for two hours. The $^{14}CO_2$ developed in the reaction was trapped by a folded filter paper containing 200 μl Hyamine (NEN) in a plastic center well. The well was transferred to a scintillation vial containing 10 ml Dimiscint (National Diagnostics). The samples were counted for 10 minutes after dissipation of the initial chemiluminescence.

Monoamine oxidase-A. Monoamine oxidase-A was assayed with our modification of the method of Gabay *et al.* [13]. Duplicate 25 μl aliquots of a 10% homogenate in 0.32 M sucrose were incubated at pH 7.4 at 37°C for 30 minutes with 4 nmoles 5-hydroxy (side chain-2-14C) tryptamine (50 mCi/nmole SpA, Amersham) and 36 nmoles 5-hydroxytryptamine (final concentrations). The reaction was stopped on ice with cold 2 N HCl and the radioactive product was extracted with 5 ml cold ethyl acetate. A 2 ml aliquot of the supernatant was added to 10 ml Aquasol (NEN) and counted for 10 minutes in a scintillation counter.

Protein Assay

Protein concentration was determined by the method of Lowry *et al.* [19]. Aliquots containing 1–2 mg of tissue were assayed.

Statistics

All results were analyzed by one-way analysis of variance (ANOVA) followed by Student's *t* test for between-group comparisons. ANOVA statistics include control, T40%, T30%, and pair-fed groups.

RESULTS

Growth and Lifespan

Animals were followed in terms of whole body and organ weights, reproductive ability, morbidity and mortality throughout the lifespan and most of the data collected are available elsewhere [22, 27–30]. We report here briefly only on body weight, as a gross indication of health, and on mortality, as a guideline for lifespan duration. Body weights of controls (either fed Purina rat chow or the 100% tryptophan supplemented Teklad diet, ad lib) follow the well established growth curves for Long-Evans rats. Body weights of the experimental animals lagged behind [40–60%] those of controls throughout life, even after the tryptophan-deficient animals were transferred to the complete diet at two years of age. At about 30 months of age, the body weights of controls dropped rapidly (30% in six months) while the weights of the experimental animals were only slightly reduced. The sharp decline in weight among controls accompanied a marked in-

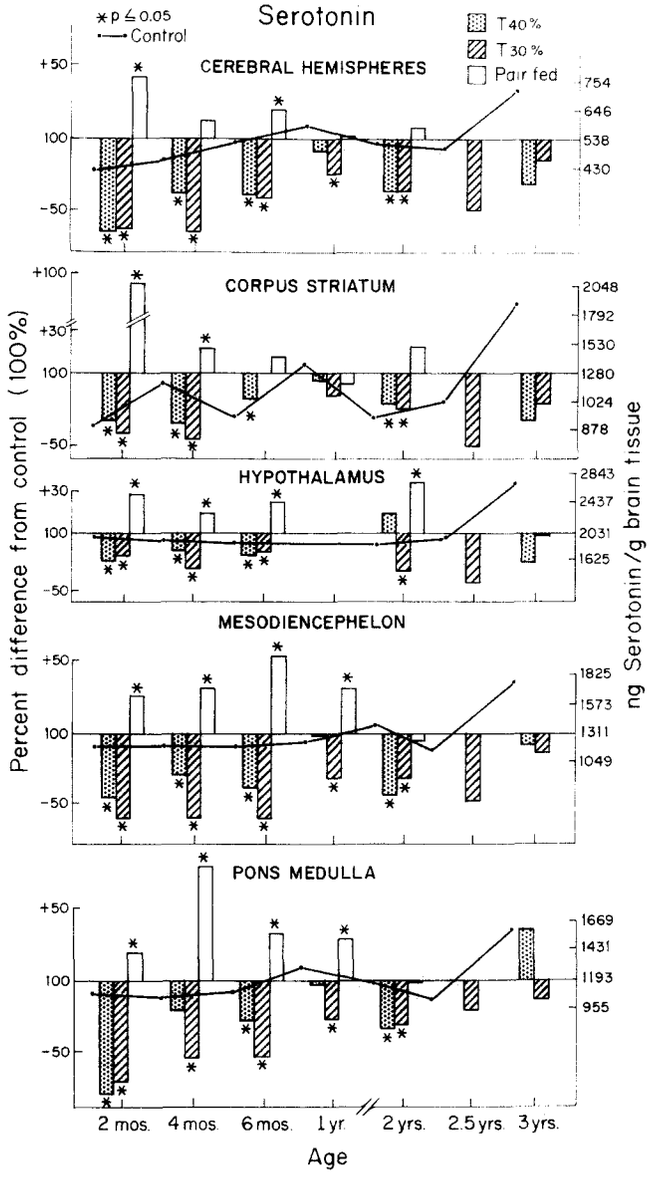

FIG. 1. Diet- and age-associated changes in brain serotonin levels. Tryptophan-restricted diets (containing 40% or 30% optimal tryptophan) were fed from weaning to 2 years of age, thereafter normal (optimal tryptophan) diet was fed. Pair-fed animals were given normal diet equal in weight to the tryptophan-restricted diet consumed by their counterparts. The connected lines indicates the mean control levels (ng/g brain tissue) of serotonin at different ages. Bars indicate the per cent difference from control level (100%) of serotonin in cerebral hemispheres, corpus striatum, hypothalamus, mesodiencephalon, and pons-medulla at ages from 2 months to 3 years. Each bar represents 5 animals except beyond 2 years when only 1–2 animals are represented. Standard errors have been omitted for clarity. Significant differences ($p<0.05$) are indicated with *.

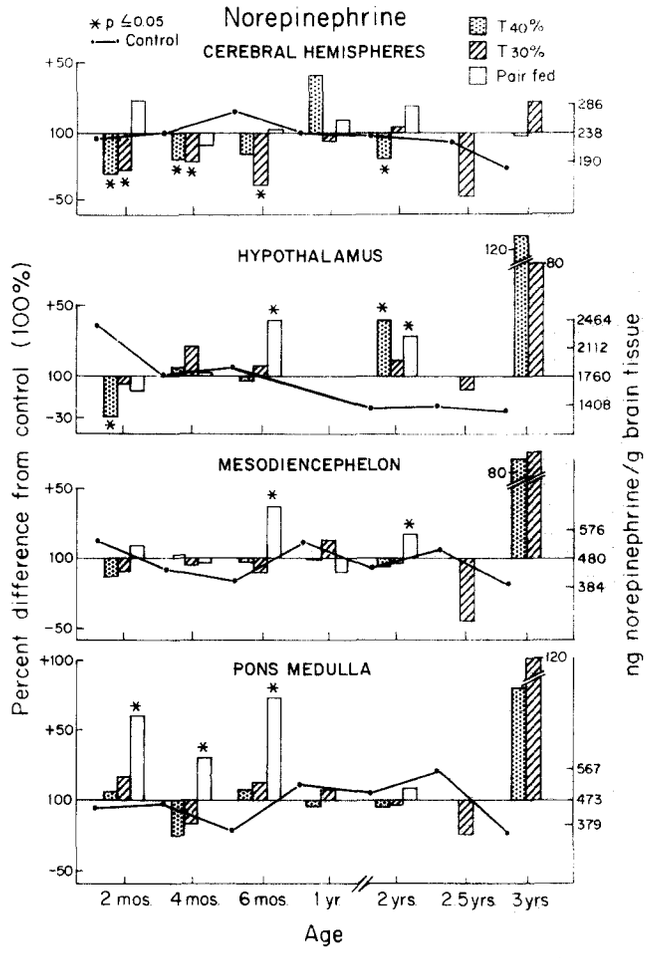

FIG. 2. Diet- and age-associated changes in brain norepinephrine levels. Tryptophan-restricted diets (containing 40% or 30% optimal tryptophan) were fed from weaning to 2 years of age, thereafter normal (optimal tyrptophan) diet was fed. Pair-fed were fed normal diet equal in weight to the tryptophan-restricted diet consumed by their counterparts. The connected line indicates the mean control levels (ng/g brain tissue) of norepinephrine at different ages. Bars indicate the percent difference from control level (100%) of norepinephrine in cerebral hemispheres, hypothalamus, mesodiencephalon and pons-medulla at ages from 2 months to 3 years. Each bar represents 5 animals except beyond 2 years when only 1–2 animals are represented. Standard errors have been omitted for clarity. Significant differences ($p<0.05$) are indicated with *.

crease in mortality: from no mortality at one year to 18% at 18 months and 91% at 36 months. The median length of life of control rats in this study was about 23.5 months, the same as reported for male SPF Fisher 344 rats [40]. In the Tryptophan-restricted rats, the mortality was high at early ages, 25% for T40% and 36% for T30% at one year, however, the mortality rate decreased thereafter. Maximal lifespan in these restricted animals were longer (1,347 days in T40% and 1,527 days in T30%) as compared to controls (1,246 days). In pair-fed groups, mortality was less than for controls at two years of age when this portion of the study was concluded.

Brain Monoamine Levels

In controls (Purina chow or 100% tryptophan supplemented Teklad diet) monoamine levels were the same and, therefore, values shown in Table 2 are the mean of both groups. Serotonin levels, generally, remained unchanged until advanced age when they increased in the hypothalamus, mesodiencephalon and pons-medulla (Fig. 1). In the cerebral hemispheres, levels increased up to one year of age, declined between one and two-and-a-half years and then in-

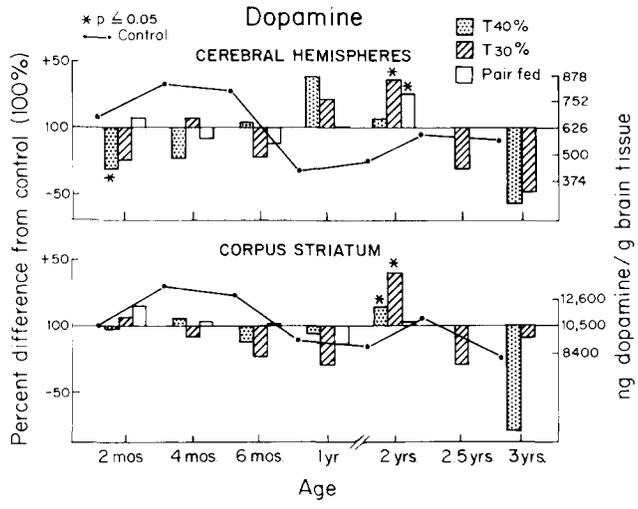

FIG. 3. Diet- and age-associated changes in brain dopamine levels. Tryptophan-restricted diets (containing 40% or 30% optimal tryptophan) were fed from weaning to 2 years of age, thereafter normal (optimal tryptophan) diet was fed. Pair fed rats were given normal diet equal in weight to the tryptophan-restricted diet consumed by their counterparts. The connected line indicates mean control levels (ng/g brain tissue) of dopamine at different ages. Bars indicate the percent difference from control level (100%) of dopamine in cerebral hemispheres and corpus striatum at ages from 2 months to 3 years. Each bar represents 5 animals except beyond 2 years when only 1–2 animals are represented. Standard errors have been omitted for clarity, significant differences ($p<0.05$) are indicated with *.

creased to three years. In the striatum, serotonin levels increased progressively from two to 12 months, and then again at three years. Norepinephrine and dopamine levels decreased with age in all areas where measured (Figs. 2, 3). This decrease was not uniform: norepinephrine in the hypothalamus decreased by 50% between two months and two years but remained practically unchanged in the cerebral hemispheres over the same age period. Dopamine levels increased between two and six months, a period when the axonal endings are still proliferating in this region, and then decreased in old rats.

In the tryptophan-deficient animals, serotonin levels were lower (except in the 3-year old pons-medulla) than controls at all ages and in all brain areas (Fig. 1). ANOVA revealed marked differences in serotonin for all brain areas at early ages. These differences were less, but still significant, at later ages when fewer areas were affected. For example in the cerebral hemispheres at two months, $F(3,21)=55.75$; at four months, $F(3,17)=19.42$; at six months, $F(3,21)=15.33$; at one year, $F(3,17)=2.28$; and at two years, $F(3,27)=8.41$. In the hypothalamus the values of two months were, $F(3,21)=18.07$; at four months, $F(3,17)=11.51$) at six months, $F(3,19)=5.92$; at one year not assayed; and at two years, $F(3,27)=2.02$. The degree of reduction, greater in some areas than in others (e.g., at two months, 80% in pons-medulla, 20% in the hypothalamus), is related in most cases to the severity of restriction, the most restricted having the lowest serotonin content. Dietary rehabilitation at approximately 24 months of age did not significantly alter the reduced serotonin levels. At three years, some regions showed a small degree of recovery and the pons-medulla definitely increased in serotonin in the T40% animals.

The effects of tryptophan dificiency were more variable on catecholamine than on serotonin content (Figs. 2, 3). Dopamine levels were lower or unchanged at younger ages in tryptophan-deficient animals, then significantly higher at two years of age (for example, in cerebral hemispheres, $F(3,27)=5.97$) and dropped below control levels again at later ages (Fig. 3). The most striking changes were a reduction of norepinephrine in the cerebral hemispheres especially at early ages (two months, $F(3,21)=8.01$, four months, $(3,17)=3.32$, six months, $F(3,21)=4.50$, one year, $F(3,17)=4.03$, two years, $F(3,27)=4.55$). Sporadic changes were observed in other areas, such as the mesodiencephalon rich in adrenergic neurons (at one year, $F(3,20)=23.71$) and in the hypothalamus, norepinephrine responsive tissue for release of neurohormones (at six months, $F(3,19)=5.56$). Norepinephrine markedly increased in the hypothalamus, mesodiencephalon and pons-medulla at 3 years of age after food rehabilitation (Fig. 2).

A striking difference between control and pair-fed animals was reflected in serotonin levels which were all significantly higher in pair-fed than controls (Fig. 1). Similarly, norepinephrine levels in these animals were increased overall, although the increase was not as uniform or marked as that of serotonin (Fig. 2), Dopamine levels were essentially unchanged or increased. (Fig. 3).

Monoaminergic Enzyme Activity

Studies of the various metabolic enzymes were carried out as permitted by the available amounts of tissue and activity of the individual enzymes. Due to these limitations all enzymes could not be studied in all areas at all ages.

In controls, tyrosine hydroxylase activity reached adult values by two months of age and remained unchanged up to three years of age except for large increases at this late age in the corpus striatum and pons medulla in the few remaining animals (Table 3). Tryptophan restriction did not significantly alter the enzyme activity at any age. A general overview of the effects of pair-feeding showed an increase in tryosine hydroxylase, significant in the cerebral hemispheres and mesodiencephalon at four months of age, in the corpus striatum at one and two years and in the hypothalamus at one year, the only age studied.

Tryptophan hydroxylase activity was assayed in fresh tissue in those brain regions most involved in serotonergic transmission [26]. The activity was highest at two months in the mesodiencephalon and pons-medulla, at one year in the cerebral hemispheres and declined thereafter (Table 4). Despite the marked decrease in serotonin levels in the tryptophan-deficient animals the enzyme activity was unaltered.

When measured at two years of age (Table 5), the activity of monoamine oxidase-A, involved in serotonin catabolism, was unchanged by the tryptophan deficiency and associated low levels of brain serotonin. Pair feeding did not change the monoamine-oxidase A activity.

DISCUSSION

The picture of the aging brain emerging from current and earlier data as well as those of other investigators [21, 24, 31–36] is one of age, region, and neurotransmitter specificity. While catecholamine levels decline progressively with aging in most brain areas, serotonin levels, initially decreasing in the cerebral hemispheres, remain unchanged or increase in

TABLE 3

TYROSINE HYDROXYLASE ACTIVITY IN BRAINS OF FEMALE LONG-EVANS RATS

	Tyrosine Hydroxylase (nmoles/hr/mg protein)					
Age	2 months	4 months	6 months	1 year	2 years	3 years
Cerebral Hemispheres						
Control (10)*	0.46 ± 0.07†	0.39 ± 0.08	0.58 ± 0.08	0.51 ± 0.11	0.44 ± 0.04	0.46
T40% (5)	0.65 ± 0.26	0.51 ± 0.09	0.70 ± 0.21	0.71 ± 0.15	0.65 ± 0.07	0.73
T30% (5)	0.60 ± 0.14	0.37 ± 0.11	0.79 ± 0.17	0.94 ± 0.22	0.61 ± 0.04	0.64
Pair-fed (5)	0.36 ± 0.02	0.77 ± 0.08‡	0.73 ± 0.03	0.84 ± 0.06‡	0.48 ± 0.09	—
Mesodiencephelon						
Control	0.61 ± 0.06	0.53 ± 0.07	0.68 ± 0.07	0.84 ± 0.05	0.60 ± 0.05	0.54
T40%	0.58 ± 0.09	0.66 ± 0.10	0.64 ± 0.08	0.83 ± 0.04	0.73 ± 0.09	0.31
T30%	0.46 ± 0.12	0.48 ± 0.04	0.65 ± 0.05	0.61 ± 0.14	0.66 ± 0.09	1.10
Pair-fed	0.81 ± 0.09	1.12 ± 0.08‡	1.17 ± 0.12‡	1.21 ± 0.24	0.59 ± 0.03	—

	Corpus Striatum			Pons Medulla			Hypothalamus
	1 year	2 years	3 years	1 year	2 years	3 years	1 year
Control	3.85 ± 0.25	4.02 ± 0.34	6.72	0.23 ± 0.03	0.18 ± 0.01	0.40	0.67 ± 0.05
T40%	5.52 ± 0.99	5.05 ± 0.38‡	10.76	0.33 ± 0.04	0.18 ± 0.01	0.30	0.59 ± 0.04
T30%	3.65 ± 0.29	4.32 ± 0.52	8.93	0.18 ± 0.02	0.19 ± 0.02	0.34	0.58 ± 0.05
Pair-fed	5.90 ± 0.46‡	6.69 ± 0.65‡	—	0.26 ± 0.02	0.18 ± 0.01	—	0.98 ± 0.07‡

*Numbers in ( ) indicate number of animals. Two animals for 3-year-old group.
†Average ±SE.
‡$p<0.05$ between control and experimental animals.

most brain areas at advanced age [32]. The relative susceptibility to aging of brain catecholamines as opposed to the relative stability of brain serotonin is supported by morphologic studies which show a loss of catecholaminergic neurons and/or alterations in their structure with aging while serotonergic neurons seem less affected [8]. If, as postulated recently [5], catecholamines stimulate the synthesis of choline, the acetylcholine precursor, then, with aging, reduction in brain catecholamines may be responsible in part, together with the regional loss of cholinergic neurons [3,11], for the progressive cholinergic deficits in specific brain areas. The resulting imbalance, perhaps, more than a global neurotransmitter reduction may be instrumental in inducing functional neurologic and behavioral deficits of the elderly. Certain characteristics of the serotonergic pathways [2] may help explain their apparent stability into old age: (a) specialized contacts with persistently dividing glial cells [7,17] provide metabolic [15,18] support for the aging neurons; (b) multiple modes of transmission (synaptic contact, extrasynaptic diffusion, small attachment plaques) minimize the consequences on neurotransmission of the age-related reduction in dendritic domain [10,25]; and (c) ability to sprout subsequent to neurotoxic (and perhaps age-related) lesions is greater than that of the other monoaminergic neurons [4]. Further support for the preservation of brain serotonin levels until late age is provided by the sustained efficiency of metabolic enzyme activity reported here and the ability to accumulate serotonin after inhibition of catabolism by MAO blockade [32].

Long-term, dietary tryptophan restriction from weaning to old age reduces serotonin levels in most brain areas proportionally to the severity of the restriction. Without entering in the controversy of the source of brain tryptophan and consequently brain serotonin levels [14, 24, 39], the present

TABLE 4

TRYPTOPHAN HYDROXYLASE ACTIVITY IN BRAINS OF FEMALE LONG-EVANS RATS

	Tryptophan Hydroxylase (nmoles/hr/mg protein)		
Age	2 months	1 year	16–18 months
Cerebral Hemispheres			
Control (10)*	0.48 ± 0.06†	0.70 ± 0.07	0.60 ± 0.01
T40% (5)	—	0.44 ± 0.11	0.55 ± 0.04
T30% (5)	0.43 ± 0.05	0.56 ± 0.12	—
Mesodiencephelon			
Control	3.41 ± 0.37	1.09 ± 0.15	1.38 ± 0.05
T40%	—	0.91 ± 0.14	1.28 ± 0.11
T30%	2.45 ± 0.38	1.08 ± 0.43	—
Pons Medulla			
Control	2.16 ± 0.31	1.68 ± 0.22	1.55 ± 0.05
T40%	—	1.47 ± 0.33	1.46 ± 0.16
T30%	2.10 ± 0.42	2.20 ± 0.62	—

*Numbers in ( ) indicate number of animals.
†Average ±SE.

data show that dietary restriction of this amino acid induces generalized, severe and persistent reduction in brain serotonin levels; this is specific for serotonin and for tryptophan restriction. Indeed, the pair-fed animals, although retarded in growth, show brain serotonin levels—as well as catecholamines—normal or elevated, an observation in agreement with reports that acute food deprivation increases brain tryptophan [12]. The manipulation of naturally occur-

TABLE 5

MONOAMINE OXIDASE-A ACTIVITY IN BRAINS OF TWO-YEAR-OLD FEMALE LONG-EVANS RATS

		Cerebral Hemispheres	Corpus Striatum	Meso-diencephelon	Pons Medulla
Control	(10)*	78.4 ± 1.1†	70.9 ± 2.4	89.4 ± 3.7	67.1 ± 1.8
T40%	(5)	79.6 ± 0.7	72.8 ± 2.9	90.9 ± 2.1	65.0 ± 1.6
T30%	(5)	81.3 ± 1.5	65.4 ± 0.7	96.2 ± 1.7	72.8 ± 1.6
Pair-fed	(5)	75.7 ± 3.9	71.3 ± 3.7	89.4 ± 1.9	70.4 ± 2.5

*Numbers in ( ) indicate number of animals.
†Average ±SE, nmoles/hr/mg protein.

ring dietary constituents that are precursors of neurotransmitters has been advocated for the treatment of a number of brain disorders including senility. A much used precursor, although its efficacy is controversial, is choline, or lecithin. Such administration enhances regional (e.g., hippocampus) acetylcholine synthesis and is stated to reduce the cognitive deficits (e.g., memory loss) characteristic of the elderly, particularly those affected by senile dementia of the Alzheimer type [3,11]. Because of serotonin many physiological actions (e.g., endocrine, behavioral), it may be anticipated that manipulation of brain serotonin may influence a number of physiological parameters. Indeed, topical (in the hypothalamus) or systemic administration of serotonin agonists or antagonists as well as dietary tryptophan restriction may modify the timetable of reproductive development and aging [30], may mediate memory and learning [38], may prolong thermoregulatory competence [28], and increase the lifespan [29]. Whether these actions resulting from dietary or pharmacologic serotonin manipulations are due directly to the changes in brain serotonin levels, or the accompanying reduction in food intake, or indirectly result from changes in catecholamine levels remains to be elucidated. For example, caloric restriction prolongs the lifespan [20,40]. The increased catechoalminergic levels in the rehabilitated tryptophan restricted rats and the potential secondary increase in cholinergic levels [5] may explain some of the observed physiological improvement during aging.

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