Neurochemical Research, Vol. 12, No. 11, 1987, pp. 1035–1039

Effect of Aging on Monoamines and Their Metabolites in the Rat Brain

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(Accepted February 18, 1987)

Concentrations of dopamine (DA), norepinephrine (NE), serotonin (5-HT) and their acid metabolites were assayed in specific brain areas of Wistar rats of various ages. DA and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) were significantly lower in striatum and mesolimbic areas of old (24 mos) rats than young adult (3 mos), but not mature (12 mos) rats. The decrease of homovanillic acid (HVA) was significant in mesolimbic areas but not in striatum. Neither cortical NE nor its metabolite methoxyhydroxyphenylglycol sulphate (MHPG-SO_4) were significantly changed by aging. 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the brainstem showed a tendency to a decrease and increase respectively in aged animals compared with young adults, but the differences were not statistically significant. However, the ratio of 5-HIAA to 5-HT concentrations was significantly higher in aged animals. The conclusion can be drawn that, in these brain areas, DA is more vulnerable to aging than NE and 5-HT, the metabolism of the latter being even enhanced.

KEY WORDS: Aging; rat; brain; monoamines.

INTRODUCTION

It is becoming more and more evident that the effects of age on brain neurotransmitters vary in the different systems and regions and are often controversial (see reviews in 1–6). It has been reported that the content and turnover of dopamine decrease with age particularly in the striatum and mesolimbic areas (7–13), but these changes were sometimes variable or absent (13–17). Changes of norepinephrine (7, 8, 13–15, 17–22) and serotonin (7, 11, 12, 21, 23, 24) were even less constant.

This study aimed at better clarifying the age-related neurotransmitter alterations. To obtain a fuller picture, the concentrations of dopamine (DA), norepinephrine (NE), serotonin (5-HT) and their metabolites were simultaneously assayed in various brain regions of young adult, mature, and old rats.

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EXPERIMENTAL PROCEDURE

Male Wistar rats aged 3 (young adult), 12 (mature) and 24 (old) months weighing 351 ± 8, 585 ± 19 and 693 ± 19 g respectively (means ± SEM of 10 animals for each age) were obtained from Iffa-Credo, Les Oncins, France. None of them had visible tumors. The median life of this strain is 24 months. Rats were housed at 22 ± 1°C with a 12-hr light-dark cycle (6 a.m.–6 p.m.) and allowed free access to water and a standard diet with 22% protein content. Rats were decapitated and brains quickly dissected on an ice plate, basically according to (25) with the exception of the mesolimbic areas (tuberculum olfactorium, nucleus accumbens and the septum) which were dissected as described (26).

Brain regions were rapidly frozen on dry ice and kept at −80°C until assay, within 5 days. Brain regional concentrations of dopamine (DA), homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT), and 5-hydroxyin-

0364-3190/87/1100-1035$05.00/0

doleacetic acid (5-HIAA) were determined using HPLC with electrochemical detection (27, with minor modifications to detect DA too). Every determination was made from the tissue of one rat. Tissues were homogenized in 0.2 M cold perchloric acid containing 0.8 mM sodium metabisulfite. After centrifugation (Sorvall RC5-B, 15 min), supernatants were filtered (Bioanalytical System, MF-1), degassed and injected directly into the system. This consisted of a Waters pump (model 590) equipped with Waters sample injector (U6K), 5 μm particle size column (Nucleosil 5 C_{18} with pre-column) and Spectra Physics Digital Integrator Recorder. An amperometric detector (Bioanalytical System, LC-4B) equipped with a carbon paste electrode (Bioanalytical System, TL-3) was set at 0.6 V versus an Ag/AgCl reference electrode.

Norepinephrine (NE) was assayed (28) after purification on alumina and separation on a Biophase ODS 5 μm column (Bioanalytical System). The electrode was set at 0.7 V. Standard curves of reference compounds were found to be linear over a wide concentration range (0.02–2 ng). Methoxyhydroxyphenylglycol sulphate (MHPG-SO_4) was assayed spectrofluorometrically (29).

Proteins were determined by the method of Lowry et al. (30) using bovine serum albumin for the standard curve. Data were analyzed by Duncan's multiple range test (31).

RESULTS

The concentrations of DA, HVA and DOPAC were reduced by aging both in striatum and mesolimbic areas (Table I). In striatum, the decrease in old compared to young adult rats was 26% (DA), 17% (HVA) and 16% (DOPAC), while in mesolimbic areas it was 28% (HVA), 24% (DOPAC) and 23% (DA). These differences were significant or highly significant except for striatal HVA. 5-HT was also significantly lowered by aging in striatum (−26%), but not in mesolimbic areas, whereas 5-HIAA showed a similar, insignificant increase with age in both areas.

Neither cortical NE nor its metabolite MHPG-SO_4 was significantly altered by aging (Table II). Again, 5-HT showed a tendency to decrease and 5-HIAA to increase with aging in the brainstem, but without ever reaching statistical significance.

DISCUSSION

The main findings of the present study are:

1. The content of DA and its metabolites HVA and DOPAC in DA-rich brain regions such as striatum and mesolimbic was lower in old than young rats;
2. No significant changes of NE and MHPG-SO_4 could be detected in the frontal cortex;
3. 5-HT tended to fall and 5-HIAA to rise with age in the three regions considered, striatum, mesolimbic and brainstem.

DA and its metabolites already began to decline in mature rats, but this was more evident and generally significant in the old ones, indicating that it is related more to actual aging than to maturity. They were reduced by aging not only in striatum, but also in mesolimbic areas, thus confirming our previous and other results (8, 10, 11), but not those showing no changes in the latter region (12, 13). Sex, strain or dissection differences could account for this discrepancy.

In agreement with other reports (10, 13, 15, 32), including one assessing a large number of data (33), we found that the ratios of HVA and DOPAC to DA concentrations remained constant with aging in both areas, except for DOPAC to DA in striatum which was slightly raised (data not shown). This indicates that in basal conditions DA metabolism is not reduced by aging as the remaining neurons can probably compensate for the neuronal degeneration. However, when synthesis inhibition was employed to analyze DA turnover, reports were more conflicting (8, 12, 15, 34).

The fact that the cortical NE and MHPG-SO_4 content was not significantly changed in old rats confirms the heterogeneity of the aging of neurotransmitter systems. NE was reduced with age when assayed in the locus coeruleus of various species (17, 21, 22), but, when the region contained NE projection fibers rather than cell bodies, changes were usually absent (7, 8, 11, 13, 15), except in the hypothalamus where controversial data were reported (7, 8, 12, 13, 15, 17, 20, 22) probably due to marked regional differences. In line with our present findings, no senescent decline in NE was described in human (18), rat (7, 19) and rabbit (14) cortex.

We found an age-related trend toward a decrease in 5-HT and increase of its metabolite, 5-HIAA. While most reports indicate that 5-HT does not change significantly with age (7, 11, 12, 21, 23, 24), rises in 5-HIAA have been described (12, 21, 24), although not unanimously (11). The observed tendency to an elevation of 5-HIAA and the resulting highly significant increase of the 5-HIAA to 5-HT ratio with age found in the present study (0.83 ± 0.06 in young and 1.16 ± 0.04 in old striatum; 0.55 ± 0.03 in young and 0.72 ± 0.04 in old mesolimbic; 0.81 ± 0.03 in young and 1.06 ± 0.06 in old brainstem) may reflect either stimulation of 5-

Table I. Concentrations of Dopamine (DA), Homovanillic Acid (HVA), 3,4-Dihydroxyphenylacetic Acid (DOPAC), 5-Hydroxytryptamine (5-HT), and 5-Hydroxyindole Acetic Acid (5-HIAA) in the Striatum and Mesolimbic Areas of Young Adult (3 Mos), Mature (12 Mos) and Old (24 Mos) Rats

	Striatum					Mesolimbic Areas				
Age	DA	HVA	DOPAC	5-HT	5-HIAA	DA	HVA	DOPAC	5-HT	5-HIAA
	($ng.mg^{-1}$ protein)									
Young adult	151.3 ± 5.4 (100)	6.61 ± 0.53 (100)	12.54 ± 0.70 (100)	9.13 ± 0.93 (100)	7.10 ± 0.29 (100)	35.6 ± 2.3 (100)	1.88 ± 0.20 (100)	5.41 ± 0.42 (100)	11.88 ± 0.74 (100)	6.52 ± 0.38 (100)
Mature	140.3 ± 3.6 (93)	5.97 ± 0.43 (90)	11.81 ± 0.51 (94)	8.11 ± 0.50 (89)	8.34 ± 0.51 (117)	31.8 ± 1.9 (89)	1.51 ± 0.10 (80)	4.77 ± 0.28 (88)	11.61 ± 0.78 (98)	7.08 ± 0.41 (108)
Old	112.4 ± 6.1[a] (74)	5.52 ± 0.43 (83)	10.51 ± 0.54[b] (84)	6.77 ± 0.48[b] (74)	7.79 ± 0.45 (110)	27.5 ± 0.8[a] (77)	1.36 ± 0.09[b] (72)	4.12 ± 0.22[b] (76)	10.36 ± 0.52 (87)	7.39 ± 0.33 (113)

Values are means ± SEM of 10 animals. In parentheses, the percentage of the young adult rat value. (a) $P < 0.01$ or (b) $P < 0.05$ compared to young rats (Duncan's test)

Table II. Concentrations of Norepinephrine (NE) and Methoxyhydroxyphenylglycol Sulphate ($MHPG\text{-}SO_4$) in the Frontal Cortex and of 5-Hydroxytryptamine (5-HT) and 5-Hydroxyindole Acetic Acid (5-HIAA) in the Brainstem of Young Adult (3 Mos), Mature (12 Mos) and Old (24 Mos) Rats

	Frontal cortex		Brainstem	
Age	NE	$MHPG\text{-}SO_4$	5-HT	5-HIAA
	$ng.mg^{-1}$ protein			
Young adult	5.04 ± 0.40 (100)	1.97 ± 0.10 (100)	7.21 ± 0.58 (100)	5.77 ± 0.38 (100)
Mature	6.24 ± 0.62 (124)	1.88 ± 0.13 (95)	7.00 ± 0.26 (97)	6.40 ± 0.25 (111)
Old	5.52 ± 0.58 (109)	1.93 ± 0.15 (98)	6.44 ± 0.42 (89)	6.64 ± 0.26 (115)

Values are means ± SEM of 10 animals.
In parentheses, the percentage of the young adult rat value.

HT (intra)neuronal metabolism (35) or increased monoamine oxidase (MAO) activity. Since 5-HT is a substrate of MAO A which, in contrast to increased MAO B (21, 36, 37) is unaltered (36) or even slightly reduced (37) during aging, the former hypothesis is more probable.

The conclusion can be drawn that, of the three monoamines, the DA system is especially vulnerable to aging, whereas those of NE and 5-HT are more resistant. Indeed, our study found evidence of increased 5-HT metabolism.

These results might explain not only the age-related impairment of various neurophysiological functions, but also the pathogenesis of various disorders of old age which might originate from imbalance between neurotransmitter systems (23, 38). Interestingly, this imbalance (e.g. between DA and 5-HT) is greatly enhanced in situations of increased functional demand, as that resulting from stress (33).

ACKNOWLEDGMENTS

The Authors wish to thank Dr. A Ardia and Mrs. L. Parrinello of the Farmitalia Carlo Erba Biometric Department for statistical analysis.

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