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RELATION OF SEX AND AGEING TO SEROTONIN METABOLISM IN RATS

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SUMMARY

The present study deals with serotonin (5-HT) metabolism at the central and peripheral levels in young, middle-aged and old male and female rats.

From middle age, the level of endogenous 5-HT and the uptake of [^{14}C] 5-HT were higher in the platelets of female rats than in those of male rats. By contrast, the retention of [^{14}C] 5-HT by the platelets of male rats decreased after middle age. The 5-hydroxyindoleacetic acid (5-HIAA) level in plasma was lower in males than in females, and this level increased significantly with age in males.

In brain, the tryptophan level decreased significantly with age in male rats, while the 5-HT level increased in males as well as females. The brain 5-HIAA level increased significantly with age in male rats.

These results confirm that 5-HT metabolism is modified during the ageing process in rats, and that several factors may be involved in this modification.

INTRODUCTION

Information about age-related changes in serotonergic metabolism, in both laboratory animals and men, is often contradictory. Several papers dealing with the effects of ageing on the serotonergic system fail to indicate significant modifications of the steady-state levels of serotonin (5-HT) or of the level of the metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the brain and peripheral organs in aged men and animals [1–3]. However, there has been shown to be an alteration of the metabolism of tryptophan (Trp), the precursor of 5-HT, in aged people [4], a decrease in the excretion of 5-HIAA [5], and an increase of the 5-HIAA level in the cerebrospinal fluid in senescent humans [6, 7]. Moreover, there has recently been shown to be a decrease in brain 5-HT

levels in old chickens [8] and old rats [9], an increase in 5-HT metabolism in the hypothalamus of aged rats [10], and we have previously found an increase of 5-HIAA levels in the plasma of senescent female rats [11]. These results indicate that alterations of 5-HT metabolism occur during the ageing process.

Since it has been suggested that changes in 5-HT uptake and storage in blood platelets could mirror central serotonergic modifications [12], it was of interest to study the platelet 5-HT retention as a function of age and sex. The following study deals with 5-HT platelet uptake and storage and brain 5-HT metabolism in young, middle-aged and old male and female rats.

METHODS

The determinations were performed on three groups of 20 male and 20 female Sprague–Dawley rats, 2, 10 and 18 months old. They were placed in wire-mesh cages (42 cm length, 27 cm wide, 20 cm high) in groups of 5 animals and kept in an animal room at a constant temperature (20 ± 1 °C) and humidity (52 ± 2%) with a 12-h alternating light/dark cycle. Sterile commercial food [13] and water were given *ad libitum*. No infections were noted during the experiment. All assays were done over a 30-day interval in the month of June and the animals were always sacrificed at the same time of day (10–11 a.m.) in order to avoid circadian variations of biogenic amine levels. After a slight ether anaesthesia, 5–8 ml of arterial blood were drawn from the abdominal aorta, and the brain was removed for amine analysis. Blood samples were first centrifuged at 800 *g* for 20 minutes in order to obtain platelet-rich plasma (PRP).

An aliquot of 1 ml was used to determine the uptake and release of [^{14}C] 5-HT according to the method of Lingjaerde [14]. The kinetics of 5-HT accumulation were determined in preliminary experiments performed in young rats. They were K_m = 0.2 μM, V_{max} = 32 pmoles per 10^7 platelets per min. The percentage [^{14}C] 5-HT released, determined 5, 10 and 30 minutes after the incubation had been stopped, was 7, 15 and 30%, respectively. In the present study the platelets were incubated with 0.2 μM [^{14}C]-5-HT and the release was measured at 30 minutes.

Another aliquot of 0.5 ml was used to determine the 5-HT level in the platelets and an aliquot of 0.05 ml was used to count the platelets under a phase-contrast microscope. The remaining PRP was then centrifuged at 2000 *g* for 20 minutes in order to obtain platelet-poor plasma (PPP) in which the 5-HIAA level was determined. Brain and platelet 5-HT was extracted on an Amberlite column [15]. Brain Trp and 5-HIAA contained in the effluent of the Amberlite column were extracted on a Dowex column [16].

Fluorimetric determinations were performed according to the method of Maickel and Miller [17] for 5-HT, Korf and Valkenburg-Sikkema [18] for 5-HIAA and of Bedard *et al.* [19] for Trp.

Statistical calculations were done by Student's *t*-test and by Fisher's analysis of variance. When *p* values were higher than 0.05, differences were not considered statistically significant.

RESULTS

Platelet 5-HT

Ageing did not seem to induce noticeable change in the levels of 5-HT in PRP in males or females. However, the platelet count in PRP increases during ageing in males and slightly decreases in females, so that when the level of 5-HT is related to the number of platelets, the results showed that from middle-age the platelets of female rats are richer in 5-HT than those of male rats (males *vs.* females at 10 months $F^1_{74} = 8.326, p < 0.01$; at 18 months, $F^1_{74} = 5.924, p < 0.02$) (Fig. 1).

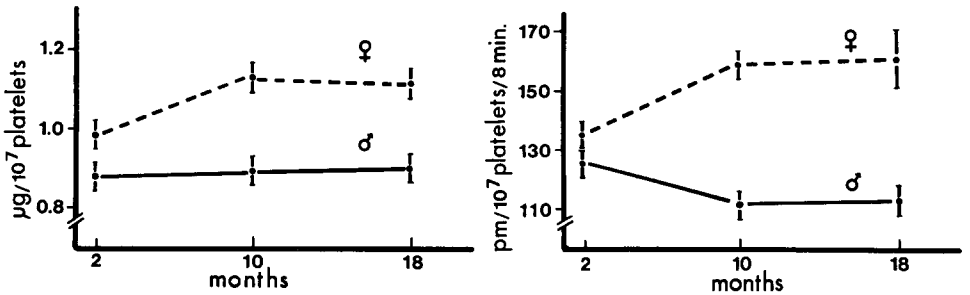

Fig. 1. Blood platelet 5-HT levels in young, middle-aged and old male and female rats. Each point represents the mean ± S.E.M. of 20 determinations.

Fig. 2. Uptake of [^{14}C] 5-HT by platelets of young, middle-aged and old male and female rats. Platelets were incubated at 37 °C with 0.2 μM [^{14}C] 5-HT for 8 minutes. Results are expressed in picomoles (pm) per 10^7 platelets per 8 min. Each point represents the mean ± S.E.M. of 20 determinations.

Uptake and release of [^{14}C] 5-HT by platelets

Ageing did not induce a noticeable change in the amount of [^{14}C] 5-HT taken up by the platelets of male rats but did induce a significant increase in the amount taken up by the platelets of female rats which correlated with age ($r = 0.278$, $F^1_{57} = 4.79, p < 0.05$). The variance analysis indicated that the uptake of [^{14}C] 5-HT became significantly higher in females than in males at middle age (males *vs.* females at 10 months $F^1_{67} = 67.5, p < 0.001$; at 18 months $F^1_{67} = 25.8, p < 0.001$) (Fig. 2).

By contrast, the percentage release of [^{14}C] 5-HT from the platelets in females did not change during the ageing process but this percentage increased significantly in males (2 months *vs.* 10 months $t = 3.859, p < 0.001$; 12 months *vs.* 18 months $t = 4.38, p < 0.001$) (Fig. 3).

This increase in release of labelled 5-HT indicates that a decrease in the mechanism of retention of the amine in the platelets occurs in male rats of middle and old age.

Plasma 5-HIAA

The 5-HIAA levels in the plasma were lower in males than in females at the age of 2 months ($t = 2.466, p < 0.02$).

No significant modification of these levels occurred during ageing in females, but they increased in males and this increase was significantly related to age ($r = 0.376$, $F^1_{40} = 6.607, p < 0.02$) (Fig. 4).

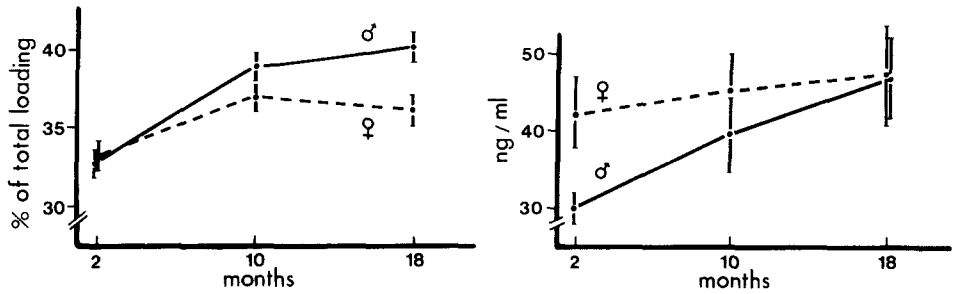

Fig. 3. Efflux of [^{14}C]5-HT from platelets of young, middle-aged and old male and female rats. Platelets were loaded with 0.2 μM [^{14}C]5-HT at 37 °C for 8 minutes. After isolation, platelets were resuspended and reincubated at 37 °C for 30 minutes. Control samples were kept in iced water under which condition no efflux occurs. The percentage decrease in platelet-bound [^{14}C]5-HT during the reincubation was taken as the measure of the efflux. Each point represents the mean ± S.E.M. of 20 determinations.

Fig. 4. Plasma 5-HIAA levels in young, middle-aged and old male and female rats. Each point represents the mean ± S.E.M. of 14 determinations.

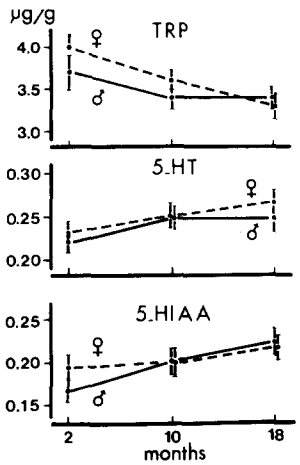

Fig. 5. Brain Trp, 5-HT and 5-HIAA levels in young, middle-aged and old male and female rats. Each point represents the mean ± S.E.M. of at least 15 determinations.

Brain Trp, 5-HT and 5-HIAA

As shown in Fig. 5, brain tryptophan levels decreased with age in female rats. This decrease was significantly related to age ($r = 0.529, F^1_{52} = 20.186, p < 0.001$).

By contrast, the brain 5-HT levels increased during the ageing process. There was a positive correlation between 5-HT levels and age in males ($r = 0.302, F^1_{56} = 5.642, p < 0.02$) as well as females ($r = 0.324, F^1_{54} = 6.356, p < 0.02$).

A slight but not significant increase in the brain 5-HIAA levels occurred during ageing in female rats. In male rats this increase was significantly related to age ($r = 0.426, F^1_{43} = 9.531, p < 0.01$).

DISCUSSION

The positive correlation between increased blood and brain 5-HIAA levels and age that we observed in male rats could be related to a change in monoamine oxidase activity. Indeed, it has been reported that in rats the activity of this enzyme increases with age in the liver and the heart [20]. However, this activity is not modified in the brain [20].

In fact, it appears that the increase in plasma 5-HIAA levels in male rats could also be related to the decrease in the 5-HT retention observed in the platelets of these animals. This hypothesis of a defect of retention of the amine by the storage sites can be extended to the results observed in the brain. Indeed, it has been suggested by several authors (see references in ref. 12) that results based on platelets could give a good evaluation of the uptake and storage of the amine in nerve terminals. So one could suggest that the increase in brain 5-HIAA level in male rats during ageing could be, at least in part, the consequence of an impairment of the storage of the amine in the nerve terminals, and investigations on brain synaptosomes remain to be made to test such an hypothesis. Indeed one can also envisage that the increase in brain 5-HT and 5-HIAA levels could be due to an increase in 5-HT turnover. Such an increase has already been reported in the hypothalamus of old rats and this change has been related to modifications in circulating sex-hormone levels [10].

Regarding the problem of the decrease of the Trp level in the brain of male rats, it seems surprising to observe in these rats an increase in 5-HT levels, since it was commonly considered that 5-HT synthesis is positively correlated with the level of Trp in brain. However, it should be recalled that the regulation of 5-HT levels is also dependent on other factors [21].

Moreover, tryptophan is as important in protein synthesis as it is in 5-HT synthesis and the decrease in Trp levels observed in old rats could also be correlated with a modification of protein synthesis. Furthermore, variations of the levels of several other free amino acids have been reported in old rats [22].

It is apparent that definitive conclusions regarding the variations of serotonergic activity in old age require numerous investigations, but the results reported in several recent papers and in the present study confirm that this activity is modified and suggest that determinations of 5-HT uptake by blood platelets and of plasma 5-HIAA level in old humans could provide fruitful information for the evaluation of serotonergic metabolism during the ageing process.

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