Activation of Vasopressinergic and Oxytocinergic Neurons During Aging in the Wistar Rat

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FLIERS, E. AND D. F. SWAAB. Activation of vasopressinergic and oxytocinergic neurons during aging in the Wistar rat. PEPTIDES 4(2) 165-170, 1983.—The activity of the hypothalamo-neurohypophyseal system (HNS) was determined in male Wistar rats from 3 to 32 months of age. Plasma levels of vasopressin (AVP) and oxytocin (OXT) were measured by means of a radioimmunoassay. In addition, the distribution of the Golgi apparatus marker enzyme thiaminepyrophosphatase (TPPase) was measured as a parameter for neurosecretory activity in the hypothalamic supraoptic and paraventricular nuclei (SON and PVN). Plasma levels of radioimmunoassayable AVP were increased in the 32-month-old animals. Plasma levels of radioimmunoassayable OXT in 32-month-old animals did not differ from the levels found in the youngest group, but were higher than in 11-month-old animals. Neurosecretory activity in the SON was similar in 3- and 32-month-old animals, whereas in the PVN neurosecretory activity was increased in the 32-month-old animals. Urine excretion decreased between 6 and 11 months of age and remained on the same level until 32 months of age. In other words, instead of a loss of HNS function as has been suggested in the literature, an increased neurosecretory activity was observed in aged rats.

Aging Vasopressin Oxytocin Neurosecretory activity Supraoptic nucleus Paraventricular nucleus Thiamine pyrophosphatase Golgi apparatus

AGING of the central nervous system is generally considered to be accompanied by both degenerative changes and cell loss, resulting in a shrinkage of the brain [26]. The changes are, however, not found to the same extent throughout the brain, but are dependent on the area and the cell type studied [14,20]. The function of the hypothalamo-neurohypophyseal system (HNS) has been suggested to diminish during the course of aging. Human plasma levels of neurophysin decrease during aging [17], and vasopressin was claimed to improve performance on memory tests in 50-65 year-old men [16]. Furthermore, oxytocin (OXT) injections resulted in a prolongation of lifespan in rats [4].

A study of changes during aging in the "neurosecretory neurons" of the HNS has some unique advantages as compared with studying "conventional" neurons. Vasopressinergic and oxytocinergic neurons project via the hypothalamo-neurohypophyseal tract to the neural lobe of the pituitary, where they release vasopressin (AVP) and oxytocin (OXT) into the bloodstream [2]. The activity of these peptidergic neurons can be followed by assaying vasopressin and oxytocin in the peripheral circulation. In addition, vasopressin and oxytocin neurons project into a large number of brain areas [6, 7, 25]. In such regions they terminate by means of synapses that cannot be distinguished morphologically from conventional amnergic or aminoacid containing synapses [8].

The older literature unanimously states that the changes during aging in the rat HNS indicate a decrease in neurosec-

retory activity. The amount of neurosecretory material was reported to be reduced in the posterior lobe of rats older than 24 months, as revealed by Golgi staining [22]. Urinary excretion of vasopressin and plasma levels of this peptide were claimed to decline with advancing age in the rat, while the turnover of water would increase [31]. Recently, decreased amounts of vasopressin were observed in the hypothalamic and extrahypothalamic areas of old rats [11], while an impaired dehydration-induced response of the HNS in old rats was reported [28]. In addition, AVP content in the hypothalamus and plasma levels of AVP were reported to be decreased in aged Sprague-Dawley rats [33]. Also some of the behavioral deficits in old rats have been attributed to age-dependent changes in the secretion of AVP [9]. Furthermore, recent immunocytochemical studies revealed age-related changes in the neurosecretory cells, although the direction of the change in activity was not clear. A decreased staining intensity of magnocellular neurons was reported, especially in neurons containing oxytocin, the paraventricular nucleus (PVN) being more severely affected than the supraoptic nucleus (SON) [32]. Other investigators, however, reported an increase in the number of neurons that stained darkly for neurophysin in the SON, while the PVN remained unaltered [19].

The aim of the present study was to reinvestigate the suggested decline in basal functional activity of the rat HNS during aging, using a combination of techniques. Plasma levels of AVP and OXT were measured by means of radioimmunoassay (RIA). As a measure for neurosecretory
activity the distribution of the Golgi apparatus was determined in the SON and PVN. This organelle is involved in packaging neurosecretory granules and can be labelled by the enzyme thiamine-pyrophosphatase (TPP-ase). The distribution of this marker increases with enhancement of neurosecretory activity, e.g., during water deprivation, pregnancy, parturition, lactation, castration and during the oestrous [15,29].

METHOD

Animals

Adult male Wistar rats (CPB:WU, 5 groups of different ages, n=98) were obtained from TNO-CPB (Zeist, The Netherlands). The youngest (3-month-old) animals were delivered directly from TNO-CPB, whereas the older animals (6–32-month-old) were kindly donated by Dr. H. Rigter (Or ganon, Oss, The Netherlands), who had used them as untreated controls in short-lasting behavioral experiments. They were housed in groups of no more than 5, under constant temperature and humidity, and received tap water and food (Hope Farms, Woerden, The Netherlands) ad lib. Lights were on between 7 a.m. and 7 p.m.

General Procedures

The 24-hr urine excretion was measured in metabolism cages, during which period the animals received tap water ad lib, but no food. This was because only the old animals often spoiled large amounts of food in the cages, which disturbed the urine measurements. The animals were weighed and decapitated between 09:00 and 11:30 a.m., after which the brains were dissected immediately and weighed. One animal which, at 26 months of age, was found to have a pituitary tumor, was excluded from further study. Blood was collected from the trunk in chilled and heparinized plastic tubes and immediately processed for RIA. All assays were set up in duplicate. Details on the extraction procedure and RIA for AVP and OXT have been described elsewhere [10]. The AVP and OXT-RIA had a negligible cross-reaction of 0.03% with OXT and AVP, respectively. The between-assay coefficients of variation of extracted samples in our assay procedure were 13.9% for AVP and 8.6% for OXT. The intra-assay coefficients of variation were 15.7% and 10.2%, respectively. The lower limit of detection was 1.5 pg/ml for AVP and 11.1 pg/ml for OXT. If the values were below the limit of the assay, the detection limit was used as an actual value.

Histochemistry

Animals used for this part of the study were selected at random. Hypothalamic areas were excised, fixed in 4% glyoxal in cacodylate buffered sucrose (4°C) for 24 hr, and washed in cacodylate buffered sucrose. After a washing period of 2×24 hr with constant agitation the tissue was frozen in liquid Freon-12 at −80°C. Cryostat sections (16 μm) of the SON and the PVN were used for the staining of the Golgi apparatus marker enzyme TPP-ase, which was quantified as described before [15,29]. In brief, the distribution of TPP-ase deposits in the SON and the posterior magnocellular part (cf., [30]) of the PVN was determined by means of a Zeiss Hennig ocular, combined with an oil immersion (100×) objective. The distribution of the enzyme deposits was expressed as the percentage of the 100 points of the Hennig
oculor hitting the Golgi apparatus in each SON or PVN section. In each SON 16 sections were counted and in each PVN 8 sections were counted per animal.

Statistics

Plasma AVP and OXT levels. Since the observed distribution of values of radioimmunoassayable AVP and OXT showed too much deviation from a normal distribution, non-parametric Kruskal-Wallis one-way analysis of variance (including correction for ties) was used to assess significance of differences. If p-values were less than 0.05, individual groups were compared using the Mann-Whitney U test (0.05 level of significance).

Weight, urine excretion, neurosecretory activity. The distribution of individual values over the various age groups was tested by means of analysis of variance. If the p-values were below 0.05, the Newman-Keuls method was used to test differences between pairs of means (0.05 level of significance).

RESULTS

Body Weight, Brain Weight and Urine Excretion

Body weight increased significantly (p<0.05) from 3 months up to 11 months, after which a decrease was observed until 32 months of age (Fig. 1). Brain weight continuously increased (p<0.05) up to 11 months, whereas differences between 11, 26 and 32-month-old animals were not significant (see Fig. 2). Urine excretion (total 24 hr volume±standard error of the mean (SEM) decreased from 28.4±2.4 ml at 3 months to 17.7±2.3 ml at 32 months, the differences between successive age groups being only significant (p<0.05) between 6 and 11 months (Fig. 3).

Plasma Levels of AVP and OXT

At 32 months of age, the levels of radioimmunoassayable AVP (range: 1.5–59.5 pg/ml) were significantly higher than those at 3 (p<0.03) and 11 (p<0.02) months (range: 1.5–41.7 pg/ml and 1.5–13.3 pg/ml, respectively), while the latter groups did not differ from each other (Fig. 4). Also the levels of radioimmunoassayable OXT were highest in the oldest age group (range: 11.1–108.8 pg/ml), while the lowest values were measured in the 11-month group (range: 11.1–31.6 pg/ml). The values for 3 months (range: 11.1–100.9 pg/ml) and for 32 months did not differ significantly from one another, but the 32-month value was significantly (p<0.01) higher than the 11-month value.

Neurosecretory Activity

Five age groups were studied for the determination of the distribution of TPP-ase. Lead sulphide deposits could be discerned as brown, perinuclear bands in the PVN and SON neurons of all animals. The TPP-ase staining of the Golgi apparatus could easily be distinguished from the staining in nucleoli and blood vessels which was not counted. The SON neurons in the various age groups did not show any striking
difference in the size of the Golgi apparatus. In the PVN of most 32-month-old animals, however, the staining was more intense and mostly covered all of the perikaryon, in contrast to the perinuclear localization observed in the younger age groups (Fig. 5).

Quantitative determination of the distribution of the enzyme deposits (Fig. 6) in the PVN revealed a non-significant trend to decline up to 26 months of age. In the PVN the percentage of positive hits in 32-month-old animals was significantly ($p < 0.05$) higher than in all the younger age groups, which did not differ significantly from each other. Regarding the SON, 32-month-old animals did not differ significantly from 3- and 6-month-old animals. However, the mean percentage of hits in 11- and 26-month-old animals was significantly lower than in 32-month-old animals ($p < 0.05$).

**DISCUSSION**

The observations concerning the decreased human plasma neurophysin levels between 51 and 60 years of age [17] and the various animal experimental observations suggesting degenerative changes of the rat HNS during aging (see introductory paragraphs) indicated that the rat might be a useful animal model to study the mechanisms underlying the hypothesized decline of HNS function in man. However, up till now most observations on the HNS itself have been based upon methods that do not allow any conclusions to be drawn about the nature of the changes during aging (i.e., inhibition vs. activation). Therefore we determined changes in the distribution of the Golgi apparatus, which has been proved to be a measure for neurosecretory activity. The observation of an inverse relationship between the amount of intraneuronal lipofuscin accumulation and the size of the Golgi apparatus in the aged rhesus monkey [1] made this parameter, in addition, of special interest to gerontology.

The fact that the cells of the PVN appeared to be activated at 32 months of age was a surprise in view of the earlier reports in the literature, suggesting decreased neurosecretory activity. Although there is no neuron loss in the SON and PVN of senescent male Sprague-Dawley rats [21], data on cell numbers in the SON and PVN of aged Wistar rats do not exist. However, the activation of the HNS is not to be considered a compensatory mechanism for cell loss, in view of the enhanced plasma levels of AVP in the 32 month old animals.

In the present study the changes in intracellular distribution of the Golgi apparatus in the SON and PVN were related...
FIG. 6A. Percentage of thiamine pyrophosphatase-positive hits in the supraoptic nucleus of rats from 3 to 32 months of age. Bars indicate the mean for each age group, vertical lines indicate the SEM (n represents the number of animals in each group). *Significantly (p<0.05) higher than 11 and 26 month group.

FIG. 6B. Percentage of thiamine pyrophosphatase-positive hits in the paraventricular nucleus of rats from 3 to 32 months of age. Bars indicate the mean for each age group, vertical lines indicate the SEM (n represents the number of animals in each group). The oldest age group shows an increase in the distribution of the Golgi apparatus enzyme. *Significantly (p<0.05) higher than any other age group.

to changes in plasma levels of radioimmunoassayable AVP and OXT. The increased neurosecretory activity in the PVN at 32 months proved to be concomitant with a clearcut increase in the plasma level of radioimmunoassayable AVP. Data appearing during the preparation of this manuscript, showing a rise in the plasma level of vasopressin during aging, both in humans and the rat [12], support the finding of an activation of the HNS during aging. However, the particular strain of rats was not mentioned. These findings contrast with earlier bioassay data of other investigators [31], who reported a fall in the plasma level of AVP in 23-30 month-old male Wistar rats. In male Fischer 344 rats, basal plasma levels of AVP were reported to be similar in 3- and 30-month-old animals [28]. Some of the discrepancies may be explained by strain differences and by the fact that no generally accepted definition of "the aged rat" exists in the literature. If the 50% survival age is used as a definition, 30 months of age should be considered as "old" for most laboratory rat strains [5]. However, since the rate of aging is highly dependent on dietary and other environmental factors [18], even 30-month-old rats of the same strain are not necessarily comparable with respect to age-related variables. Our "aged" animals had been fed ad lib and had been housed in groups of approximately 5 animals, which may be considered to be "environmental enrichment" in comparison with the (standard) single housed condition (e.g. [13]). The differences in AVP plasma levels between our 11- and 32 month-old animals cannot be explained by differences in environmental factors, since all animals older than 3 months had been obtained from Organon (Oss) and had been exposed to the same conditions.

It is not clear how the enhanced HNS activity is caused. It might be secondary to changes in the target organ for vasopressin, the kidney. Impairment of the urinary concentrating ability of the kidney and a decrease in the vasopressin dependent cAMP generation has been reported in 24-month-old Fisher 344 rats [3]. We were unable to confirm earlier statements on the occurrence of diabetes insipidus in the old rat [31], as we found no differences in urine excretion between 11 and 32 months of age. Still, our animals may have developed a decreased kidney sensitivity to vasopressin, maintaining the urinary excretion at the same level as the 11-month-old animals by higher AVP levels. However, it is unexpected in this condition that the PVN shows the most pronounced activation. In young animals, osmotic stress results in increased activity in the SON as well as in the PVN, the SON reacting earlier and stronger than the PVN [15]. A possible explanation for the increased neurosecretory activity mainly in the PVN may be differential age-related alterations in the pattern of affrent innervation of the SON and the PVN. An age-related decline in the pattern of noradrenergic innervation of magnocellular elements in the SON of the rat was reported [27]. The PVN, however, was not taken into consideration.

The observed activation of the PVN in the aged rat may have consequences for central functions in which AVP and OXT are supposed to be involved, as the central and peripheral release of neurohypophysial hormones seem to be coupled under various conditions [22]. In a preliminary experiment Tj. B. Van Wimersma Greidanus found in a single sample of pooled cerebrospinal fluid (CSF) of 3 aged rats (24 months of age) an AVP level of approximately 65 pg/ml, which is about three times higher than the level found in 3-month-old rats (personal communication). In this context it
is of interest to note that aged animals of the same strain showed remarkably few deficits on memory tests [23].

Future research will be directed towards the question if there are any consequences of the observed activation of the PVN for the extrahypothalamic peptidergic pathways. Furthermore, we shall focus on the human HNS in order to establish whether the changes related to aging in the human and rat HNS are paralleled.

REFERENCES


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