Activation of Vasopressin Neurons in the Human Supraoptic and Paraventricular Nucleus in Senescence and Senile Dementia

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SUMMARY

A recent study has shown that vasopressin (AVP) cells in the human supraoptic (SON) and paraventricular (PVN) nuclei increase in size after 60 years of age, suggesting that AVP production is increased in senescence. In the present study, the same brain material was used for the determination of nucleolar size in immunocytochemically identified AVP and oxytocin (OXT) neurons as an additional parameter for peptide production.

A strong correlation was found between nucleolar size and cell size, both in AVP and OXT neurons. Nucleolar size of AVP but not of OXT neurons increased significantly in senescence. Observations in brains from patients with senile dementia of the Alzheimer type (SDAT) were commensurate with their ages. These results strongly support the hypothesis that AVP neurons in the SON and PVN are activated in old age.

Key words: *Aging – Oxytocin – Senile dementia of Alzheimer type – Vasopressin*

INTRODUCTION

The supraoptic nucleus (SON) and paraventricular nucleus (PVN) are the production sites of vasopressin (AVP) and oxytocin (OXT) in the hypothalamo-

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neurohypophyseal system (HNS). These neuropeptides are known to act peripherally on diuresis (Handler and Orloff 1981), lactation and labour (Swaab and Boer 1979). Putative central functions of AVP and OXT, such as the regulation of body temperature, blood pressure and plasma osmolality and their involvement in cognitive functions (reviewed by De Wied 1983), are thought to be effectuated by extrahypothalamic pathways, in which these neuropeptides probably act as a neurotransmitter (reviewed by Buijs 1982).

Both peripheral and central functions in which AVP is supposed to be implicated are frequently affected during aging and in senile dementia of the Alzheimer type (SDAT) (Bengele et al. 1981; Balmagiya and Rozovski 1983; Jolles and Hijman 1983). Some authors have postulated that the HNS degenerates in old age (Rodeck et al. 1960; Legros 1975; Turkington and Everitt 1976; Watkins and Choy 1980; Sladek et al. 1981; Li and Nagai 1984), but more recent work suggests that, on the contrary, the activity of AVP cells increases in senescence (Helderman et al. 1978; Legros et al. 1980; Frolkis et al. 1982; Fliers and Swaab 1983; Kirkland et al. 1984).

Recently, an increase in cell size was found in immunocytochemically identified AVP but not OXT neurons of the human SON and PVN after the age of 80 yr, which is suggestive of an increased peptide production from this age onwards (Swaab et al. 1984a; Fliers et al. 1985). However, although the correlation between cell size and peptide production is well established in adulthood (Zambrano and De Robertis 1968; Kalimo 1975), one may question whether this relationship also persists in senescence since, among other reasons, pigments, known to accumulate in many types of aging nerve cells (Mann and Yates 1974), may cause an enlargement in cytoplasmic volume. Therefore, nucleolar size, which has been shown to be a good parameter for neurosecretory activity in these neurons (Edström et al. 1961; Ifft 1962; Zambrano and De Robertis 1968; Russell 1983), was determined in the present study.

MATERIALS AND METHODS

Material and staining procedures

Brains from 30 patients ranging in age from 10 to 93 years and including 4 patients who had clinically and pathologically been diagnosed as SDAT cases were obtained at autopsy. For further details on the patient material the reader is referred to Fliers et al. (1985).

Following fixation in 10% formaldehyde, 6 μ m paraffin sections were cut transversally. From each subject, two adjacent sections from the central part of the PVN and dorsolateral SON were selected for immunocytochemical staining with AVP and OXT antiserum, respectively, and subsequently counterstained in order to visualize the nucleoli, using the following protocol (all antisera were diluted in phosphate-buffered saline (PBS), pH 7.4): (a) sections were deparaffinized in xylene (2 $\times$ 10 min) and hydrated in graded ethanol series (2 min per step), followed by washing in PBS (2 $\times$ 10 min); (b) preincubating with 10% goat serum, containing 0.5% Triton (10 min); (c) incubating with either OXT-preadsorbed AVP antiserum (No. 126-OXT, 1 : 200) or AVP-preadsorbed OXT antiserum (No. O-I-V-AVP 1 : 400), containing 0.5% Triton

(overnight at 4 °C); (d) washing in PBS (2 × 10 min); (e) incubating with goat anti-rabbit serum (Betsy, 1 : 50; 30 min); (f) washing in PBS (2 × 10 min); (g) incubating with peroxidase–antiperoxidase (PAP) (1 : 1000) (30 min); (h) washing in PBS (2 × 10 min); (i) rinsing in 0.05 M Tris–HCl, pH 7.6; (j) incubating with 0.5 mg/ml 3,3'-diaminobenzidine (DAB) in 0.05 M Tris–HCl, pH 7.6, containing 0.01% H₂O₂ (10 min); (k) rinsing in distilled water; (l) incubating with gallocyanin chrome alum (overnight at 37 °C) (cf. Sandritter et al. 1966); (m) rinsing in 96% alcohol, containing 1% HCl; (n) dehydrating and embedding in Entellan.

Morphometrics and statistics

Measurements were made using a Calcomp 2000 digitizer in combination with a Zeiss microscope fitted with an oil-immersion Plan 100 × objective and 12.5 × plan oculars. Nucleolar projection area was determined for all immunocytochemically stained neurons in the SON and PVN that contained a nucleolus in the section. In order to determine the precision of the measurements 25 nucleoli were measured twice and 1 nucleolus was measured 10 times on consecutive days, revealing a relative error (i.e. standard error of the mean (SEM) expressed as percentage of mean nucleolar diameter) of 1.6% and 1.0%, respectively.

The mean number of AVP nucleoli measured per patient was 79.9 ± 6.4 (mean $\pm$ SEM) in the SON and 58.5 ± 8.0 in the PVN. The mean number of OXT nucleoli measured per patient was 12.2 ± 4.0 in the SON and 40.7 ± 3.7 in the PVN. Mean nucleolar diameters per patient were calculated from the measured nucleolar projection areas. In addition, this parameter was estimated from the unfolded values using a deconvolution procedure (cf. Weibel 1979), correcting for nucleoli being cut and appearing in the section as profiles (see Discussion).

Effects of age, sex, postmortem delay and duration of fixation on nucleolar size were tested by means of analysis of variance (ANOVA). If *P* values were below 0.05, differences between pairs of means were tested using the Student–Neuman–Keuls multiple range test (SNK, 0.05 level of significance).

Differences between nucleolar size values of SDAT patients and 8 age- and sex-matched controls were tested by means of the Mann–Whitney U-test (MW, two-tailed, corrected for ties, at a 0.05 level of significance). Correlation coefficients were calculated using the Pearson rank correlation (0.05 level of significance).

RESULTS

The gallocyanin-stained nucleoli were visible as round, sharply bordered, homogeneously dark blue structures. The nuclei appeared as unstained structures with some distinct, blue little lumbs. The brown DAB deposit of the immunocytochemical staining in the cytoplasm was well preserved (Fig. 1).

Mean nucleolar diameter of AVP cells did not change significantly until 80 yr of age, but in the oldest age group (80–100 yr) a significant (*P* < 0.03) increase was found, both in the SON and in the PVN. No significant change with aging was found in mean

Fig. 1. Alternating sections from the central part of the PVN of a 72 yr old female, stained for AVP (A) and OXT (B) and counterstained with gallocyanin. Note the absence of cross reaction, cell Nos. 1-5 being stained in A and not in B and vice versa for the cell Nos. 6-8. Also note the sharply bordered nucleoli (arrows). Bars represent 50 and 10 μ m in the low magnification photomicrograph and in the insert, respectively. III = 3rd ventricle; b = blood vessels.

Fig. 2. Mean nucleolar diameter of vasopressin (AVP) and oxytocin (OXT) cells in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) as a function of age. There is a significant effect of age on mean nucleolar diameter of AVP cells (ANOVA, $P < 0.03$ in the PVN and $P < 0.02$ in the SON), values in the 80–100-year-old group being significantly higher than in the 20–80-year-old groups in the PVN and the 40–80-year-old groups in the SON (SNK, $P < 0.05$), as indicated by the asterisks. Mean nucleolar diameter of OXT cells does not change significantly with aging (ANOVA, $P > 0.70$ in the PVN and $P > 0.40$ in the SON). Bars indicate mean values per 2 decades, vertical lines indicate SEM, n representing the number of brains examined in each age group. Open circles represent values from SDAT patients.

nucleolar diameter of OXT cells ($P > 0.40$) (Fig. 2). Note the relatively small number of OXT cell nucleoli measured in the SON.

In patients with SDAT, mean nucleolar diameters of AVP and OXT cells were not significantly different from age- and sex-matched controls ($P > 0.30$).

Sex, postmortem delay and duration of fixation did not significantly influence mean nucleolar diameter ($P > 0.31$, $P > 0.07$ and $P > 0.50$ respectively).

A significant correlation between individual mean nucleolar diameter and previously measured mean cellular profile area was found for AVP cells in the SON and PVN, as well as for OXT cells in the PVN ($r > 0.70$, $P < 0.001$), but not for the small number of OXT cells in the SON ($r = 0.47$; $P > 0.10$).

DISCUSSION

Recently, an activation of AVP cells in the human brain after 80 yr of age was hypothesized, using cell size as a parameter for peptide production (Swaab et al. 1984a;

Fliers et al. 1985). The aim of the present study was to further test this hypothesis, using nucleolar size as a measure for AVP and OXT production. The use of this parameter would, in contrast to cell size, avoid possible bias from cytoplasmic accumulation of pigments in old age.

Nucleolar size has been shown to be a reliable parameter for peptide production in these neurons in many activating conditions, such as osmotic stress (Edström et al. 1961; Ifft 1962), lactation (Russell 1983) and following castration (Zambrano and De Robertis 1968). In addition, nucleolar size has been shown to be proportional to both nucleolar and cytoplasmic ribonucleic acid content in these neurosecretory cells (Edström and Eichner 1958; Mann et al. 1981).

In order to be able to interpret the present results, the measured structures were assumed to be the complete nucleolar spheres and not sectioned profiles, since the mean nucleolar diameters, as calculated from the unfolded values did not differ from the observed, uncorrected, mean diameters. This might be explained by the observation that in paraffin sections the nucleolus is more compact than its surroundings and will, therefore, generally not be split when sections are cut, but rather remain intact in either of two adjacent sections (Koningsmark 1970).

The significantly increased mean nucleolar diameter of AVP neurons in senescence reinforces the idea that the previously observed increase in cell size is indeed at least in part due to an enhanced neurosecretory activity rather than to cytoplasmic storage of "age" pigments such as melanin or lipofuscin. Hence, the present results support the postulated activation of AVP cells in the SON and PVN in senescence.

It is not known at present whether or not this activation is a compensatory mechanism for AVP cell loss in senescence. Although no significant decrease in AVP cell density was observed during aging (Fliers et al. 1985), definite proof for the absence of cell loss can only be obtained by determination of the total AVP cell number in the SON and PVN. On the other hand, a number of recent studies have reported elevated basal plasma levels of AVP in the aged rat and in man (Frolkis et al. 1982; Rondeau et al. 1982; Fliers and Swaab 1983; Goddard and Davies 1984; Kirkland et al. 1984; Phillips et al. 1984), which argues, in the absence of any age-related differences in AVP catabolism (cf. Robertson and Rowe 1980), in favor of an increased AVP secretion from the hypothalamus.

In our material, nucleolar sizes as measured in brains from patients with SDAT were found to be within the normal range for their age group. This finding is in contrast with earlier data of Mann et al. (1981), who reported a reduction in nucleolar size in SON and PVN neurons in demented patients. However, the number of demented patients in both studies was small, while also the use of immunocytochemistry in the present study may have led to different results.

Several mechanisms might underlie the activation of AVP neurons in the HNS in old age, one possibility being that it is of primarily central origin. In this respect it is of interest that changes in the afferent monoaminergic innervation pattern of the SON and the PVN have been described during aging (Sladek et al. 1983). Such mechanisms could possibly underlie the idiopathic syndrome of inappropriate ADH secretion (SIADH) which has been suggested to be related to senescence (Goldstein et al. 1983).

In our patient material, however, no indications for SIADH could be obtained from the laboratory data reported in the medical reports.

A peripherally induced activation of the HNS on the basis of age-related changes in the kidney is also a possible explanation. The decreased urine concentrating ability observed in man (Rowe et al. 1976) has been attributed to intrinsic renal factors (Miller and Shock 1953; Phillips et al. 1984). Subsequently, it was shown that the ability to generate cAMP in the renal papilla upon administration of AVP is decreased in old rats (Beck and Yu 1982; Goddard et al. 1984). More recently, the immunocytochemical staining of AVP binding sites in the renal tubuli of the aged rat was found to be diminished as compared with young animals (Swaab et al. 1984b; Ravid et al., in preparation) pointing to a primarily renal cause for the activation of AVP neurons in the SON and PVN. However, further studies are needed to clarify the mechanism by which the activation of AVP cells in old age is effectuated.

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