CHANGES IN VASOPRESSIN NEURONS AND FIBERS IN AGING AND ALZHEIMER'S DISEASE: REVERSIBILITY IN THE RAT

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SUMMARY

The neuropeptide vasopressin (VP) is released from the neurohypophysis into the circulation where it acts as antidiuretic hormone on the kidney. In addition, VP is present in nerve cells and fibers in several areas in the rodent and primate brain where it acts as a neurotransmitter or neuromodulator.

In the human brain a marked decrease in total cell number and VP cell number was observed in senescence in the suprachiasmatic nucleus, the hypothalamic nucleus regulating circadian rhythms. This degeneration was even more pronounced in Alzheimer's disease (AD) and might be related to the disturbances in sleep-wake cycle and endocrine rhythms which occur in this condition. No degenerative changes were observed with aging or in AD in the human hypothalamo-neurohypophyseal system (HNS); on the contrary, total cell numbers remain unaltered and the VP cells in this system are activated in senescence, probably in compensation for decreased sensitivity of the kidney to VP. It is proposed that this activation may prevent degeneration of the VP cells in the HNS.

The extrahypothalamic VP innervation in the male rat brain was shown to be diminished in senescence in a number of areas. This innervation, which was previously shown to depend on plasma levels of sex-steroids, could be restored in a number of brain structures by subcutaneous testosterone administration to senescent male rats for one month. Reversibility of changes in VP innervation in the senescent rat brain through peripheral testosterone supplementation might open new possibilities for the development of therapeutic strategies in age-related disorders of the central nervous system.
INTRODUCTION

The neuropeptides VP and oxytocin (OT) are produced by neurons in the hypothalamic supraoptic (SON) and paraventricular (PVN) nuclei, which project to the neurohypophysis from where the peptides are released into the bloodstream (Bargmann, 1949). VP acts as antidiuretic hormone on the kidney (Handler and Orloff, 1981) and has vasopressor properties (Cowley and Barber, 1983), whereas OT is involved in labor and lactation (Swaab and Boel, 1979). When, in the early sixties, De Wied showed an impairment in cognitive function in rats following hypophysectomy which could be reversed by VP injections, the hypothesis was put forward that VP could modulate memory processes by acting on the brain as a hormone on its target organ. This hypothesis was supported by the observation that VP analogues which lack antidiurectic or vasopressor activity produced similar results [for a review see (De Wied, 1983)]. When Legros (1975) found evidence of a deficiency of neurohypophyseal hormone release in men between 50 and 60 years of age and subsequently reported hat intranasally administered VP improved memory in men aged 50-65 years (Legros et al., 1978) it was proposed that decreased VP secretion might account, at least partly, for age-related impairments in cognitive function (De Wied and Van Ree, 1982). However, later trials with VP administration to elderly and demented patients have yielded inconsistent results (for a review see [Jolles, 1986]).

HYPOTHALAMO-NEUROHYPOPHYSEAL SYSTEM

Early reports on the activity of the HNS in aging rats supported a functional impairment of VP secretion in senescence (Friedman et al., 1956; Turkington and Everitt, 1976). However, more recent studies indicate that VP release from the pituitary is increased instead of decreased in senescence in various rat strains (Frolikis et al., 1982; Fliers and Swaab, 1983; Miller, 1985; Ravid et al., 1987; Goudsmitt et al., 1988a). When Legros extended his measurements of blood levels of neurophysin (part of the precursor of VP and OT) in human subjects, he observed that the decrease in men aged 50-60 which he had reported earlier (see above) was followed by a secondary rise after the age of 70 (Legros et al., 1980). Others also presented evidence of increased activity and responsiveness of the HNS in human
aging (Frolikis et al., 1982; Heldereman et al., 1978; Robertson and Rowe, 1980; Kirkland et al., 1984; Phillips et al., 1984).

Morphological studies of the SON and PVN supported an activation of the HNS with aging in the rat (Fliers and Swaab, 1983; Kawashima and Kobayashi, 1982). In the human brain the size of VP neurons in the SON and PVN was shown to increase in subjects over 80 years of age, including Alzheimer patients (Fliers et al., 1985a). The size of the nucleoli of these cells was also shown to be increased in senescence and AD indicating that this enlargement is probably due to increased peptide synthesis rather than accumulation of age pigments (Hoogendijk et al., 1985). In contrast, the OT cells in the SON and PVN showed no signs of activation in senescence and AD (Fliers et al., 1985a; Hoogendijk et al., 1985), which might explain why Mann et al. (1985) who did not distinguish between VP and OT cells, found no increase in nucleolar size in the human SON and PVN in aging and in AD. Since cell numbers in the SON and PVN do not decline with aging in rat (Hsu and Peng, 1978; Peng and Hsu, 1982; Flood and Coleman, 1983; Sartini and Lamperti, 1985) or in man (Goudsmid et al., unpublished results) the observed activation of VP cells does not seem to be a compensation for cell loss. An alternative explanation was suggested by age-related changes in kidney function in rodents and humans. Renal concentrating ability diminishes with aging in rat (Bengele et al., 1981) and in man (Rowe et al., 1976). In the rat this decrease was shown to occur in spite of an increase in VP excretion (Goudsmid et al., 1988a). Recent immunocytochemical work by Ravid et al. (1987) showed a decrease in VP binding sites in the senescent rat kidney. In addition, renal responsiveness to VP was shown to be decreased in aged rats (Beck and Yu, 1982) and humans (Miller and Shock, 1953). Thus, the increased activity of the HNS with aging and in AD might well be secondary to age-related renal changes which would otherwise disrupt osmoregulation. Hence, 'VP substitution therapy' aimed at the improvement of cognitive function in elderly and Alzheimer patients (see above) was probably applied to patients whose neurohypophyseal function was not in the least deficient. This might account, at least in part, for the inconsistent results of this treatment (for a review, see Jolles, 1986).
SUPRACHIASMATIC NUCLEUS

A completely different picture of age-related changes was found in another VP cell-containing nucleus in the anterior part of the hypothalamus, viz. the suprachiasmatic nucleus (SCN) (Fig. 1).

Figure 1. Frontal section through a human hypothalamus stained immunocytochemically for VP. The VP cells in the SON and PVN are activated in senescence and AD, whereas the SCN shows marked degenerative changes. OC, optic chiasm; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; III, third ventricle.
This nucleus is considered to be the major circadian pacemaker of the mammalian brain, coordinating hormonal and behavioral circadian rhythms (e.g. see Rusak and Zucker, 1979). Age-related changes in circadian rhythms have been reported in man as well as in other species (for a review see Van Gool and Mirmiran, 1986a). Among the most prominent changes is a fragmentation of sleep-wake patterns in senescence (Van Gool and Mirmiran, 1983), a phenomenon that is even more pronounced in AD (Prinz et al., 1982). Since the human SCN is hardly recognizable in conventionally stained material (Lydic et al., 1980), immunocytochemical staining of VP was used in order to make morphological investigation of this nucleus possible in human material.

A marked decrease in SCN volume, VP cell number and total cell number (Fig. 2) was found in subjects aged 80-100 years, while in Alzheimer patients these changes were even more dramatic (Swaab et al., 1985).

![Bar Chart](image)

**Figure 2.** Total cell number in the SCN shows a marked decrease after 80 years of age which is even more pronounced in AD. DEM, Alzheimer's disease.
Although the volume and total cell number of this nucleus were not found to be decreased in the senescent rat brain (Peng et al., 1980; Roozendaal et al., 1987), a marked decrease in immunocytochemically identified VP and vasoactive intestinal polypeptide (VIP)-containing cells was demonstrated in aged rats (Roozendaal et al., 1987; Chee et al., 1988). Since the integrity of the SCN has been shown to be directly related to the expression of its pacemaker properties (Pickard and Turek, 1983), the observed degenerative changes in this nucleus with aging and in AD might constitute an anatomical substrate for disturbances of circadian rhythmicity under these conditions.

DIFFERENTIAL CELL LOSS: A HYPOTHESIS

The data on cell numbers in various structures in the human hypothalamus presented above show a striking diversity. On the one hand the SCN shows a marked cell loss in senescence and AD; on the other hand, total cell numbers in the SON and PVN remain stable in these conditions. We propose that the activation of the VP cells in the SON and PVN might prevent degenerative changes in these nuclei. This hypothesis would be in line with the observation that ovariectomy, which is known to cause an activation of the LHRH neurons in the arcuate nucleus prevents reactive gliosis in this nucleus in senescent female rats (Schipper et al., 1981). The hypothesis that activation of neurons prevents their degeneration ('use it or lose it') is currently under investigation.

EXTRAHYPOTHALAMIC INNERVATION

The presence of VP and OT in the brain is not limited to the hypothalamus. Neuronal pathways which are immunoreactive for VP and OT have been demonstrated in a large number of areas in the rat brain where the peptides probably act as neurotransmitter or neuromodulator (Buijs et al., 1983). Extrahypothalamic VP fibers were shown to originate from VP cells in the PVN (Sawchenko and Swanson, 1982; DeVries and Buijs, 1983); SCN (Hoorneman and Buijs, 1982); bed nucleus of the stria terminalis (DeVries and Buijs, 1983) and medial amygdala (Caffe et al., 1987). Central functions mediated by VP and OT fibers may include the regulation of blood pressure (Pittman et al., 1982),
body temperature (Naylor et al., 1986), nociception (Kordower et al., 1982), avoidance behavior (Bohus et al., 1982) and maternal behavior (Van Leengoed et al., 1987).

The VP innervation of the rat brain has been shown to be sexually dimorphic (males have a higher density of VP fibers in several brain regions than females) and to depend on peripheral levels of sex-steroids (DeVries et al., 1985). Castration of adult male rats was shown to cause a decrease in VP fiber density which could be reversed by peripheral administration of sex-steroids (DeVries et al., 1985). OT innervation was shown not to depend on plasma levels of sex steroids (DeVries et al., 1986). In the senescent male rat brain a decrease in the density of VP fibers was observed which was particularly pronounced in those brain areas where VP innervation is dependent on sex steroids (Filers et al., 1985b). Since plasma testosterone levels decrease progressively with age in the rat (Ravid et al., 1987; Kaler and Neaves, 1981), it was proposed that the age-related decrease in central VP innervation might be due to decreased plasma testosterone levels (Filers et al., 1985b). We tested this hypothesis by giving 33-month-old male Brown-Norway rats a subcutaneous implant with testosterone which elevated plasma testosterone upto the level of young animals. After one month the immunocytochemically stained VP and OT innervation of these animals was compared with the innervation in sham-treated young and senescent controls. The results showed that VP innervation was indeed restored in testosterone-treated aged rats in the ventral hippocampus, the ventral tegmental area, the substantia nigra pars compacta, the central grey (Fig. 3) and the locus coeruleus (Goudsmit et al., 1988b). In contrast OT innervation was not restored in any of the areas studied emphasizing the marked specificity of the effects of testosterone supplementation on VP innervation in the senescent rat brain.
Figure 3. Immunocytochemically stained VP fibers in the central grey of a young rat (a), an aged rat (b) and an aged rat following 1 month of testosterone supplementation (c). Note the decrease in VP fiber density with aging which is reversed by testosterone treatment.

Sex hormones probably stimulate VP synthesis in VP cells in the bed nucleus of the stria terminals and the medial amygdala, which project to the above brain areas (DeVries et al., 1985; Van Leeuwen et al., 1985). The bed nucleus of the stria terminalis and the medial amygdala contain high numbers of androgen and estrogen concentrating neurons (Stumpf and Sar, 1976). Thus, testosterone supplementation might cause neurites from VP cells in the bed nucleus of the stria terminalis and medial amygdala to 'fill up' again, resulting in enhanced immunocytochemical staining. Whether the restoration of VP innervation has physiological or behavioral consequences for the aged animals is currently under investigation.

The extrahypothalamic VP and OT innervation was also studied in the human brain (Fliers et al., 1986). The VP innervation in man was found to differ substantially from that in the rat. The innervation of limbic structures was scant, whereas in the locus coeruleus an extremely dense innervation was observed. No sexual dimorphism or age-related changes were observed in the VP and OT innervation in the human brain (Fliers et al., 1986). The VP innervation of the monkey brain is very similar to that in humans and also fails to show a sexual dimorphism (A.R. Caffe, personal communication). Study of the extrahypothalamic VP and OT innervation in AD is currently
in progress.

The only data concerning extrahypothalamic VP and OT in AD which are available at present concern changes in concentrations of the peptides in brain tissue and CSF. VP concentrations have been shown to be reduced in AD in several brain regions (Mazurek et al., 1986a) and in CSF (Sundquist et al., 1983; Sorensen et al., 1983; Raskind et al., 1986; Mazurek et al., 1986b), although an increase in CSF VP was also reported (Tsuji et al., 1982). Surprisingly, OT concentrations were reported to be increased in hippocampus and temporal cortex (Mazurek et al., 1987), whereas OT concentrations in CSF were found to be either reduced (Unger et al., 1971) or unaltered (Raskind et al., 1986) in AD. The interpretation of data on concentrations of neuropeptides in brain tissue and CSF is extremely difficult when no additional data on the metabolic activity of the cells of origin are available and it must be born in mind that a decrease in concentration in brain tissue might result not only from reduced synthesis, but also from increased transport, metabolic turnover or release.

The differences in VP innervation between rat and man indicate that results obtained in experimental animals cannot simply be extrapolated to human aging or AD. However, the reversibility of age-related changes in innervation by peripheral supplementation of sex-steroids, as shown in the rat, might apply to other transmitter systems in man and might open new possibilities in the development of therapeutic strategies in age-related disorders of the central nervous system. In this respect, recent studies on reduced plasma estrogen levels in postmenopausal women suffering from AD as compared with age-matched controls (Fillit et al., 1986a) and the positive effects of estradiol therapy on both affective and cognitive performance in these patients (Fillit et al., 1986b) might be of interest.

CONCLUSIONS

Recent research does not confirm the occurrence of degenerative changes in the HNS with aging and in AD as had been proposed in the past. On the contrary, this system shows an age-related activation in both rat and man. Hence,
peripheral VP administration to elderly and demented subjects in order to improve cognitive performance would not appear to be a rational therapy. Activation of the HNS might prevent degenerative changes in the SON and PVN since total cell numbers in these nuclei do not decrease with aging or in AD. In contrast, the SCN — the 'hypothalamic clock' — shows a marked degeneration in senescence which is even more pronounced in AD and which might be related to disturbances in circadian rhythms in these conditions.

Restoration of the extrahypothalamic VP innervation in the senescent rat brain by peripheral administration of testosterone indicates that age-related changes in the central nervous system need not invariably be irreversible. Application of the right stimulus (hormonal supplementation in this case) might be a useful tool in restoring neuronal function in the aging central nervous system. The question whether this principle ('use it or lose it') is also applicable to other neuronal systems using other stimuli requires further investigation. The restoration of sleep patterns in senescent rats following exposure to an enriched environment (Van Gool and Mirmiran, 1986b) might serve as an example in this regard.

ACKNOWLEDGEMENTS

The authors wish to thank Ms. T. Eikelboom for secretarial help, Mr. H. Stoffels and Mr. G. van der Meulen for preparing the illustrations and Dr. R.A. Baker for correcting the English. This study was supported by the Foundation for Medical and Health Research (MEDIGON; grant nr. 900-552-056), The Netherlands Organization for Scientific Research (NWO) and the Commission of the European Communities Concerted Action on Cellular Aging and Disease (EURAGE).

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