NEUROENDOCRINE ASPECTS OF NORMAL AND PATHOLOGICAL AGING

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INTRODUCTION

Aging of the central nervous system is accompanied by a large number of neuroanatomical, neurophysiological and neurochemical changes (1). However, the aging process does not occur in a homogenous way throughout the brain, but appears to affect some systems more severely than others (2). E.g., age-related changes in volume, cell number, neuronal size and lipofuscin content have been reported to vary widely among different structures in the human brain (3,4). The majority of subcortical structures were reported to show little change in senescence with the distinct exception of the locus coeruleus which shows a marked cell loss after 63 years of age (5). However, recent studies from our group demonstrated age-related cell loss occurs also in the human hypothalamus. Within this small area differential patterns of degeneration were observed in senescence (see below).

Hypothalamic changes in normal aging and neurodegenerative disease have not been extensively studied in the human brain, so far. In particular, no data on cell loss in this area were available. However, the presence of Alzheimer-type pathological changes in several hypothalamic nuclei in Alzheimer patients and, to a lesser extent in normal aging (6,7,8), suggested degenerative changes might occur in this part of the brain. The study of hypothalamic changes is of special interest in view of the changes in endocrine function and in circadian rhythmicity that have been demonstrated in senescence and Alzheimer’s disease (AD). E.g., plasma levels of gonadal steroids are dramatically decreased in both male and female senescence (9,10) and characteristic plasma hormone changes were observed in Alzheimer’s disease (11). Fragmentation of sleep-wake patterns is a phenomenon observed in senescence which is even more pronounced in AD (12,13). The present study focusses on changes in the anterior part of the hypothalamus in senescence and AD. Special attention was given to vasopressin (AVP) and oxytocin (OXT) neurons since changes in AVP secretion were proposed to be involved in age-related memory decline (14). Parallel studies were performed in the aged rat with special attention to the role of age-related changes in plasma levels of sex steroids on AVP and OXT secretion and on central AVP and OXT projections.
Fig. 1. Frontal section through a human hypothalamus stained immunocytochemically for AVP. The AVP 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; SON, supraoptic nucleus; III, third ventricle. Bar, 1 mm.

HYPOTHALAMO–NEUROHYPOPHYSEAL SYSTEM

The neuropeptides AVP and OXT 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 (15). AVP acts as antidiuretic hormone on the kidney (16) and has vasopressor properties (17), whereas OXT is involved in labor and lactation (18). When, in the early sixties, De Wied showed an impairment in cognitive function in rats following hypophysectomy which could be reversed by AVP injections, the hypothesis was put forward that AVP could modulate memory processes by acting on the brain as a hormone on its target organ. This hypothesis was supported by the observation that AVP analogues which lack antidiuretic or vasopressor activity produced similar results (for a review see (19)). When Legros et al. (20) found evidence of a deficiency of neurohypophyseal hormone release in men between 50 and 60 years of age and subsequently reported that intranasally
administered AVP improved memory in men aged 50–65 years (21) it was proposed that decreased AVP secretion might account, at least partly, for age-related impairments in cognitive function (14). However, later trials with AVP administration to elderly and demented patients have yielded inconsistent results (for a review see (22)).

Early reports on the activity of the HNS in aging rats supported a functional impairment of AVP secretion in senescence (23,24). However, more recent studies indicate that AVP release from the pituitary is increased instead of decreased in senescence in various rat strains (25,26,27,28,29). When Legros extended his measurements of blood levels of neurophysin (part of the precursor of AVP and OXT) 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 (30). Others also presented evidence of increased activity and responsiveness of the HNS in human aging (25,31,32,33,34).

Morphological studies of the SON and PVN supported an activation of the HNS with aging in the rat (26,35). In the human brain the size of AVP neurons in the SON and PVN was shown to increase in subjects over 80 years of age, including Alzheimer patients (36). The size of the nucleoli of these cells was also shown to be increased in senescence and AD indicating that the cellular enlargement is probably due to increased peptide synthesis rather than accumulation of age pigments (37). In contrast, the OXT cells in the SON and PVN showed no signs of activation in senescence and AD (36,37), which might explain why Mann et al. (38), who did not distinguish between AVP and OXT cells, found no increase in nucleolar size in the human SON and PVN with aging and in AD. Since cell numbers in the SON and PVN do not decline with aging in rat (39,40,41,42) or in man (Goudsmit et al., submitted) the observed activation of AVP cells does not seem to be a compensation for cell loss.

The observed changes in HNS activity might be explained by the profound changes in gonadal function that take place in both male and female aging (9,10,28,43,44). Variations in hypothalamic content of AVP and OXT were observed during the estrous cycle in rats (45) and plasma AVP levels were shown to vary with the stage of the cycle in both rats and humans (46,47). In addition, gonadectomy and subsequent hormonal substitution were shown to alter plasma AVP levels in both male and female rats (48,49). However, there is considerable disagreement on the question whether estradiol and/or testosterone stimulate or inhibit synthesis and release of AVP (48,49,50). Since an inhibition of AVP synthesis by testosterone is supported at least by part of the evidence (48,50), the question arose whether the age-related increase in HNS activity might be explained by the decreased plasma levels of gonadal hormones in senescence. This hypothesis was investigated by studying the effects of
long-term testosterone supplementation on water metabolism and AVP and OXT excretion in senescent male rats. Since no significant effects of testosterone supplementation were observed on any of the parameters studied, declining plasma testosterone levels do not appear to be the major cause of HNS activation in senescence (29). Whether declining estrogen levels affect HNS function in the female rat during aging remains to be investigated.

An alternative explanation for the activation of the HNS in senescence was suggested by age-related changes in kidney function in rodents and humans. Renal concentrating ability diminishes with aging in rat (51) and in man (52). In the rat this decrease was shown to occur in spite of an increase in AVP excretion (29). Recent immunocytochemical work by Ravid et al. (28) showed a decrease in AVP binding sites in the senescent rat kidney. In addition, renal resposiveness to AVP was shown to be decreased in aged rats (53) and humans (54). 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 (22)).

SUPRACHIASMATIC NUCLEUS

A completely different picture of age-related changes was found in another AVP cell-containing nucleus in the anterior part of the hypothalamus, viz. the suprachiasmatic nucleus (SCN) (Fig. 1). This nucleus is considered to be the major circadian pacemaker of the mammalian brain, coordinating hormonal and behavioral circadian rhythms (e.g. see (55)). Age-related changes in circadian rhythm have been reported in man as well as in other species (for a review see (56)). Among the most prominent changes is a fragmentation of sleep-wake patterns in senescence (12), a phenomenon that is even more pronounced in AD (13). Since the human SCN is hardly recognizable in conventionally stained material (57), immunocytochemical staining of AVP was used in order to make morphological investigation of this nucleus possible in human material.

A marked decrease in SCN volume, AVP 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 (58). Although the volume and total cell number of this nucleus were not found to be decreased in the senescent rat brain (59,60), a marked decrease in immunocytochemically identified AVP and vasoactive intestinal polypeptide (VIP)-containing cells was demonstrated in aged rats (60,61). Since the integrity of the SCN has been shown to be directly related
to the expression of its pacemaker properties (62), 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.

Fig. 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.

SEXUALLY DIMORPHIC NUCLEUS OF THE PREOPTIC AREA

The sexually dimorphic nucleus of the preoptic area (SDN) was first described by Gorski et al., in the rat (63). In the male rat this nucleus was shown to be 3-8 times larger than in females and this difference was shown to depend on the perinatal steroidal environment (64). The SDN might be involved in aspects of masculine sexual behavior and reproductive endocrinological functions (65,66). The SDN was first described in the human brain by Swaab and Fliers (67) and corresponds to the intermediate nucleus described by Braak and Braak (68). Study of the development of this structure in the human hypothalamus showed that
a sexual dimorphism does not arise until the 4th year postnatally, when
cellnumbers in the female SDN start to decrease, whereas the nucleus remains
stable until approximately 50 years of age in males (69). Cell numbers in the
male SDN decrease sharply after this age and in females a second phase of marked
cell loss sets in approximately around age 60 (Fig. 3) (69,70). Therefore, the
sexual dimorphism in the human SDN might not only be related to differences in
the intrauterine exposure to sex steroids, but also to postnatal differences in
hormonal function between the sexes. The sharp decreases in cell numbers in
this nucleus later on in life might be related to the dramatic hormonal changes
which accompany both male and female senescence (70). However, at present it is
not clear whether these hormonal changes would be cause or effect of the
observed cell loss in this nucleus. Cell numbers in the SDN of Alzheimer
patients were found to be within the normal range for age and sex (69).

Fig. 3. Development and sexual differentiation of the sexual dimorphic nucleus
(SDN) of the preoptic area. Around the moment of birth cell numbers in the SDN
are equal in boys (▲) and girls (○). Peak values are reached around 2-4 years
postnatally after which a sexual differentiation sets in due to declining cell
numbers in women. Cell numbers in males show a sharp decrease only after 50
years of age. In women a second period of degeneration occurs after approx. 60
years of age. For details see (69).
DIFFERENTIAL CELL LOSS: A HYPOTHESIS

The data on cell numbers in various structures in the human hypothalamus presented above show a striking diversity. The SCN shows a marked degeneration in senescence and AD and the SDN shows different degrees of age-related cell loss in males and females and does not appear to be affected in AD. In contrast, total cell numbers in the SON and PVN remain stable in these conditions. Moreover, the SON and PVN remain relatively free of Alzheimer-type neuropathological changes in AD, in contrast to neighboring structures (6,7,8). The stability of the SON and PVN with aging and in AD might be related to the activation of AVP cells in these nuclei in senescence (see above). We propose that the activation of the AVP 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 (71). The hypothesis that activation of neurons prevents their degeneration ('use it or lose it') is currently under investigation (72).

Fig. 4. Immunocytochemically stained AVP fibers in the central grey of a young rat (left), an aged rat (middle) and an aged rat following 1 month of testosterone supplementation (right). Note the decrease in AVP fiber density with aging which is reversed by testosterone treatment.

EXTRAHYPOTHALAMIC INNERVATION

The presence of AVP and OXT in the brain is not limited to the hypothalamus. Neuronal pathways which are immunoreactive for AVP and OXT have been demonstrated in a large number of areas in the rat brain where the peptides
probably act as neurotransmitter or neuromodulator (73). Extrahypothalamic AVP fibers were shown to originate from AVP cells in the PVN (74,75); SCN (76); bed nucleus of the stria terminalis (75) and medial amygdala (77). Central functions mediated by AVP and OXT fibers may include the regulation of blood pressure (78), body temperature (79), nociception (80), avoidance behavior (81) and maternal behavior (82).

The AVP innervation of the rat brain has been shown to be sexually dimorphic (males have a higher density of AVP fibers the several brain regions than females) and to depend on peripheral levels of sex-steroids (83). Castration of adult male rats was shown to cause a decrease in AVP fiber density which could be reversed by peripheral administration of sex-steroids (83). OXT innervation was found not to depend on plasma levels of sex steroids (84). In the senescent male rat brain a decrease in the density of AVP fibers was observed which was particularly pronounced in those brain areas where AVP innervation is dependent on sex steroids (85). Since plasma testosterone levels decrease progressively with age in the rat (28,43), it was proposed that the age-related decrease in central AVP innervation might be due to decreased plasma testosterone levels (85). We tested this hypothesis by giving 33-month-old male Brown-Norway rats a subcutaneous implant with testosterone which elevated plasma testosterone up to the level of young animals. After one month the immunocytochemically stained AVP and OXT innervation of these animals was compared with the innervation in sham-treated young and senescent controls. The results showed that AVP 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. 4) and the locus coeruleus (86). In contrast OXT innervation was not restored in any of the areas studied emphasizing the marked specificity of the effects of testosterone supplementation on AVP innervation in the senescent rat brain.

Sex hormones probably stimulate AVP synthesis in AVP cells in the bed nucleus of the stria terminalis and the medial amygdala, which project to the above brain areas (83,87). The bed nucleus of the stria terminalis and the medial amygdala contain high numbers of androgen and estrogen concentrating neurons (88). Thus, testosterone supplementation might cause neurites from AVP 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 AVP innervation has physiological or behavioral consequences for the aged animals is currently under investigation.

The extrahypothalamic AVP and OXT innervation in the human brain differs substantially from the rat (89). The innervation of limbic structures was found to be scant, whereas in the locus coeruleus an extremely dense innervation was
observed. No sexual dimorphism or age-related changes were observed in the AVP and OXT innervation in the human brain (89). The AVP innervation of the monkey brain is very similar to that in humans and also fails to show a sexual dimorphism (90). Study of the extrahypothalamic AVP and OXT innervation in AD is currently in progress.

The only data concerning extrahypothalamic AVP and OXT in AD which are available at present concern changes in concentrations of the peptides in brain tissue and CSF. AVP concentrations have been shown to be reduced in AD in several brain regions (91) and in CSF (92,93,94,95), although an increase in CSF AVP was also reported (96). Surprisingly, OXT concentrations were reported to be increased in hippocampus and temporal cortex (97), whereas OXT concentrations in CSF were found to be either reduced (98) or unaltered (94) 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 AVP 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 (99) and the positive effects of estradiol therapy on both affective and cognitive performance in these patients (100) might be of interest.

CONCLUSIONS

The results of the studies reviewed in the present paper indicate differential patterns of degeneration within the anterior part of the hypothalamus in senescence and AD which might be related to functional differences between the hypothalamic nuclei. Our 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. 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 causal to disturbances in circadian rhythms in these conditions. The SDN
shows different patterns of age-related cell loss in males and females. AD patient are in the range of age- and sex-matched controls for this nucleus. The relation of SDN changes to changes in gonadal function in senescence remains to be established.

Restoration of the extrahypothalamic AVP 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 (101) might serve as an example in this regard.

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