NEUROHYPOPHYSIAL PEPTIDES IN THE HUMAN BRAIN IN RELATION TO DEVELOPMENT, SEXUAL DIFFERENTIATION, AGING AND DISEASE.

Ed van Zwieten and Dick Swaab(1)
Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands.

INTRODUCTION

In human brain three neuronal systems are known to contain neurohypophysial peptides, i.e. (i) the supraoptic and paraventricular nucleus (SON and PVN respectively) and (ii) the suprachiasmatic nucleus (SCN). In addition to these hypothalamic systems (iii) the bed nucleus of the stria terminalis (BST) contains the neurohypophysial peptide vasopressin. These peptidergic systems show quite different patterns of changes in the human in relation to development, sexual differentiation, aging and disease.

SUPRAOPTIC AND PARAVENTRICULAR NUCLEUS AND ACCESSORY NUCLEI

The large neurosecretory cells of the hypothalamic supraoptic and paraventricular nucleus (SON and PVN) produce the neuropeptides vasopressin and oxytocin which are released into the blood circulation in the neurohypophysis. In addition, magnocellular oxytocin and vasopressin containing cells of the SON and PVN are co-expressing tyrosine hydroxylase, suggesting the possibility of catecholamine production (Spencer et al., 1985). The dorsolateral SON has a volume of 3 mm³ (Goudsmit et al., 1990) and the entire SON contains some 76,000 neurons (Morton, A., 1961). The PVN has a volume of 6 mm³ (Goudsmit et al., 1991) and was estimated to consist of about 56,000 neurons of which some 25,000 contain oxytocin and 21,000 vasopressin (Wierda et al., 1991; Goudsmit et al., 1991). A recent study (A.M. Neijmeijer-Leloux, unpubl. res.) showed that the vasopressin

(1)Correspondence: Prof. D.F. Swaab, Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands.
and oxytocin cell number in the SON and PVN is at an adult level as early as the second half of gestation. The SON and PVN thus seem to develop much earlier than the SCN (Swaab et al., 1990). The neurons of the SON and PVN form a population of extremely stable cells in normal aging and in Alzheimer’s disease; no loss in neurons or total cell number was observed (Goudsmit et al., 1990; Goudsmit et al., 1991; Wierda et al., 1991). The observation that no cytoskeletal alterations were found in Alzheimer patients with several antibodies in the SON (Swaab et al., 1992) is in accordance with this stability. However, in the PVN of Alzheimer patients some neuronal and dystrophic neurite staining is observed with cytoskeletal antibodies (Swaab et al., 1992).

Various observations provide evidence for the hypothesis that activation of neurons may interfere with the process of aging, and thus prolong the life span of neurons or restore their function. This hypothesis is paraphrased as 'use it or lose it' (Swaab, 1991). The SON and PVN neurons are not only metabolically highly active throughout life, but are extra activated in the course of aging as well, as can be judged from the increase in the size of the vasopressin containing perikarya (Fliers et al., 1985), nucleoli (Hoogendijk et al., 1985) and Golgi apparatus (Lucassen et al., unpubl. res.), and the enhanced plasma levels of vasopressin (Frolkis et al., 1982). Similar activation of vasopressin neurons was observed in the aged rat (Fliers et al., 1983; Goudsmit et al., 1988) and is probably due to a loss of vasopressin receptors in the kidney during aging (Ravid et al., 1987).

Recently, we observed a 50% reduction in the number of oxytocin expressing PVN neurons in Prader-Willi syndrome, a 40% reduction in AIDS and a 20% reduction in Parkinson’s disease (Purba et al., unpubl. res.). It remains to be determined what the functional implications of these changes are, e.g. in terms of autonomic regulation, eating behavior and metabolism. Concerning the finding of decreased numbers of oxytocin neurons in Prader-Willi syndrome, characterized by enormous obesitas, it is interesting that centrally projecting oxytocin pathways are thought to inhibit food intake (Olson et al., 1991).

In contrast to the SON, the PVN does not only contain magnocellular vasopressin and oxytocin neurons, but also parvocellular ones that project to central brain regions or to the median eminence. Examples of the latter type of neurons are the corticotropin releasing hormone (CRH) neurons. In the human PVN they are not localized in a well-defined subnucleus as they are in the rat, but are spread all over the PVN, except for the most rostral part. Another property of CRH neurons in the PVN is that they co-express vasopressin when activated. This occurs in increasing numbers of CRH neurons in the process of aging, suggesting an activation of the hypothalamo-pituitary-adrenal axis (Raadsheer et al., unpubl.
Familial hypothalamic diabetes insipidus is transmitted as an autosomal dominant gene. Affected individuals have low or undetectable levels of circulating vasopressin and suffer from polydipsia and polyuria, but they respond to substitution therapy with exogenous vasopressin or analogues. Urine production may amount to some 20 liters per day. Members of a Dutch family suffering from this disease appeared to have a point mutation in one allele of the affected family members, based upon a G to T transversion within the neurophysin encoding exon B (Bahnsen et al., 1992). In a Japanese diabetes insipidus family a G to A transition has been described in the same exon (Ito et al., 1991). Some of the few postmortem histological observations in other families with hereditary hypothalamic diabetes insipidus point to severe neuronal death in the SON and PVN in case of familiar hypothalamic diabetes insipidus (Braverman et al., 1965; Nagai et al., 1984; Bergeron et al., 1991). This situation is quite different from that in the Brattleboro rat, where the mutant neurons seem to remain intact.

SUPRACHIASMATIC NUCLEUS

The suprachiasmatic nucleus (SCN) is a small structure (0.25 mm³) that is considered to be the major circadian pacemaker of the mammalian brain, coordinating hormonal and behavioral circadian rhythms (Ruzak and Zucker, 1979). In conventionally thionine-stained sections the human SCN cannot be recognized with certainty. For this purpose immunocytochemical labelling of the nucleus is necessary (Swaab et al., 1990). The shape of the human SCN is sexually dimorphic, i.e. more elongated in women and more spherical in men, but the cell number and volume are similar in both sexes (Swaab et al., 1985). Neurons that are immunoreactive for vasopressin, VIP, neuropeptide-Y and neurotensin are present in the SCN in a particular anatomical organization (Mai et al., 1991; Moore, 1992). Typical for the human SCN, as compared to monkeys and other animals, are (1) the very large population of neurotensin cells and (2) the large population of NPY neurons obscuring a geniculo-hypothalamic tract - if such a tract is present in the human brain at all (Moore, 1992). At birth the SCN contains some 13% of the vasopressin-expressing neurons and 20% of the total cell number found in adulthood. Subsequently, cell numbers rise to maximum values around 1-2 years postnatally, after which they decrease gradually to some 50% of these numbers in adulthood (Swaab et al., 1990). Recent observations have revealed a marked seasonal variation in the volume and cell number of the human SCN in relation to
the variations in photoperiod (see Fig. 1.). Values were twice as high in autumn as in summer (Hofman and Swaab, 1992). Similar circadian fluctuations were observed in the SCN of young adults (Hofman and Swaab, unpubl. results).

Figure 1. Seasonal variations in the volume of the suprachiasmatic nucleus (SCN) (A) and the number of vasopressin immunoreactive neurons in the SCN (B). Subjects were grouped into 4 annual periods of equal length based on time of death. The seasonal periods are based on a photoperiodic division of the year, i.e. Winter: 306°-35°, midpoint 21 Dec.; Spring: 36°-125°, midpoint 23 Mar.; Summer 126°-215°, midpoint 22 Jun.; Autumn 216°-305°, midpoint 21 Sep. Data are represented as means ± S.E.M.. The SCN shows a striking seasonal variation with low values in the summer and higher values in the other periods of the year (*P < 0.05; ***P < 0.001; Kruskal-Wallis multiple comparisons test (Conover, 1980).
Since fetal circadian rhythms are generally not observed during the immediate postnatal period and develop gradually - over several weeks to months postnatally (Honnebier et al., 1989), it is generally believed that the fetal rhythms are predominantly driven by the mother. The postnatal development of various overt rhythms is paralleled by a strong increase in the number of vasopressin-expressing neurons (Swaab et al., 1990). On the other hand, based upon observations in squirrel monkeys (Reppert, 1992) and the presence of temperature rhythms in some 50% of the preterms (Mirmiran and Kok, 1991) the human fetus may already be capable of expressing some endogenous circadian rhythmicity. In addition, melatonin receptors are apparent in the SCN area as early as the 18th week of gestation (Reppert, 1992).

Recent morphometric analysis of the SCN revealed in ten homosexual men that the volume of this nucleus was 1.7 times as large as that of a reference group of 18 male subjects, and that it contained 2.1 times as many cells (Swaab and Hofman, 1990). It might be that the programmed postnatal cell death, usually occurring from 13-16 months after birth onwards (Swaab et al., 1990a), is limited in homosexual men. It is not yet clear what the functional implications of this finding might be, although there are various indications that the SCN is involved in aspects of sexual behavior and reproduction (Swaab et al., 1987).

Age-related changes in circadian rhythms have been reported in man as well as in non-human species. A fragmentation of sleep-wake patterns occurs in senescence, a phenomenon that is even more pronounced in Alzheimer’s disease (Witting et al., 1990). In Alzheimer’s disease the disruptions of the circadian rhythms are often so severe that they are even thought to contribute to the mental decline, as well as often leading to hospital admission of the elderly. For this reason, the total SCN cell numbers and numbers of vasopressin expressing neurons were determined during aging and in Alzheimer’s disease. A marked decrease was found in SCN cell number in subjects of 80-100 years of age, while in Alzheimer’s disease these changes were even more dramatic (Swaab et al., 1985; Swaab et al., 1987). In this respect it is important to note that both the retina and the optic nerve, which provide direct and indirect input to the SCN, show degenerative changes in Alzheimer’s disease. In addition to degenerative changes, Alzheimer patients are generally exposed to less light than their age-matched controls. As a result, both the input of the visual system to the SCN and the SCN itself (Witting et al., 1990) seem to be seriously affected in Alzheimer’s disease. The exact contribution of each of these components to circadian disturbances has yet to be investigated. Preliminary Japanese observations showed that behavioral disturbances diminish in Alzheimer patients after exposure to bright light for two hours per morning.
THE EXTRA-HYPOTHALAMIC VASOPRESSIN SYSTEM

With respect to neurohypophyseal peptides, the bed nucleus of the stria terminalis (BST) is the only thusfar known extra-hypothalamic cellgroup in human brain which contains the neurohypophyseal peptide vasopressin (Fliers et al., 1986). In the rodent, the BST and medial amygdala give rise to a sexually dimorphic vasopressin system that is under the influence of testosterone (De Vries et al., 1984; Goudsmit, 1991).

However, the human vasopressin system is different from that in the rat, in that it is only sparsely innervated with vasopressin fibers. In contrast, brainstem nuclei such as the locus coeruleus (LC), parabrachial nucleus and the nucleus tractus solitarius are more densely innervated with vasopressin fibers in human than in rat (Van Zwieten, unpubl.res.). Moreover, studies of the extra-hypothalamic vasopressin system in the human brain have not yielded any clear sex difference or a significant change with aging. One of the most densely innervated brain regions, as far as vasopressin is concerned, is the LC which is located bilaterally in the dorsal pontine brainstem at the ventrolateral edge of the fourth ventricle. It consists of a distinct cluster of noradrenaline (NA) containing neurons and is in primates, because of the presence of neuromelanin, clearly visible without staining. Because of its extensive efferents through the entire brain (Jones, 1991), the LC may be involved in general processes like emotion, level of vigilance and sleep-wake cycle (Saper, 1987). Such processes are compromised in neurodegenerative diseases like Alzheimer's disease. In this condition the main symptoms are primarily attributed to an impaired function of frontal and temporal cortex and hippocampus (Mann and Yates, 1986). However, in addition several subcortical afferent projection systems are affected in Alzheimer's disease, one of them being the LC. Changes in the LC in Alzheimer's disease are obvious from the decreased NA content in projection regions of the LC (Palmer et al., 1987). Furthermore, recent studies show an extensive neuronal loss in the LC, using different markers for visualisation of NA neurons, i.e. neuromelanin (Bondareff et al., 1982), tyrosine-hydroxylase (Chan-Palay and Asan, 1989) or dopamine-β-hydroxylase (Iversen et al., 1983). This neuronal loss appeared to be topographically distributed, the rostral part of the LC being most, the medial part less and the caudal part the least affected (Chan-Palay and Asan, 1989). In rat it is reported that the efferent pathways of the LC have a spatial distribution within the nucleus, i.e. the anterodorsal neurons of the LC project mainly to the cortex, while ventrally located neurons project to the caudal parts of the brain (Loughlin et al., 1986b). In Alzheimer's disease the loss of rostral neurons in the LC may thus be related to cortical impairment.
Pilot experiments in our laboratory showed a stable vasopressin innervation in the region of the LC in which the highest density of neuromelanin-containing neurons were present, i.e. the caudal region (Fliers, pers. comm.). Further studies in our laboratories showed that, in accordance with literature, a decrease in number of neuromelanin containing neurons was observed in the rostral part of the LC. The vasopressin innervation in this part of the LC was however not affected (Van Zwieten, unpubl. res.). This observation suggests that there may be no major direct functional contacts between neuromelanin containing noradrenergic neurons and vasopressin fibers.

Recently a pronounced vasopressin innervation was found in the human parabrachial nucleus (PB), more than the moderate vasopressin innervation in rodents (Van Zwieten, unpubl. res.; Buijs, 1978). The PB is thought to play a role in autonomic processes and taste perception which was found to be sexually dimorphic in rodents (Di Lorenzo and Monroe, 1990). In idiopathic Parkinson’s disease the number of catecholaminergic neurons in the PB is significantly decreased, which may be related to autonomic disturbances in this condition (Goto and Hirano, 1991).

The NTS is another nucleus in the human brain in which only recently a dense vasopressin innervation could be detected (Van Zwieten, unpubl. res.), again more intens than in rodents (Van Leeuwen et al., 1986). This finding parallels the presence of neurophysin in the human brain in this region (Sofroniew et al., 1981), which is derived from the same precursor molecule as vasopressin. It has already been found by radioimmunoassay that substantial levels of vasopressin are present in this region in the human brain (Jenkins et al., 1984). This nucleus is involved in relaying visceral sensory information, i.e. concerning circulatory haemodynamics, gastric and hepatic sensory systems, to the hypothalamic PVN and SON, thus affecting vasopressin and oxytocin release to the blood (Raby and Renaud, 1989).

Concluding, it is clear that in the different stages of life and diseases, the various vasopressin and oxytocin neuronal and fiber systems of the human brain show differential changes, if such systems in the human are studied by procedures allowing microscopical resolution, chemical characterization and a good estimation of their neuronal numbers and activity stage in the various conditions.

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