The vasopressin containing neuron in the human brain; changes during ageing and senile dementia

D F SWAAB, E FLIERS and B FISSER
Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ, Amsterdam, The Netherlands

Introduction
Immunocytochemistry is used increasingly as a marker for specific cells or cell groups. Lately this technique has also been applied to the human brain. This paper gives an example of the combination of immunocytochemical and morphometrical technique as applied to (a) the vasopressin-containing cells of the supraoptic and paraventricular nuclei (SON and PVN) in order to distinguish them from oxytocin-containing cells, and (b) on the suprachiasmatic nucleus (SCN) so as to visualize this area in the human brain.

Vasopressin (AVP) and oxytocin (OXT) neurons project as classical neurosecretory neurons from the paraventricular and supraoptic nuclei (PVN and SON) to the neurohypophysis, where AVP and OXT are released into the bloodstream. These peptides are involved in diuresis, lactation and labour. In addition, vasopressin and oxytocin neurons from various nuclei project to a large number of brain areas, where they terminate by means of synapses that cannot be distinguished morphologically from aminergic or amino acid-containing ones. These extrahypothalamic projections are thought to be the anatomical basis for the hypothesized central functions of AVP and OXT, such as temperature regulation, osmotic and blood pressure regulation and their involvement in memory. These functions are supposed to be frequently disturbed during ageing and dementia, where a decreased production of AVP is presumed to occur. In the present study, we have measured cell size as a parameter for peptide production in the PVN and SON by means of a digitizer (MOP system).

Figure 1. Mean cellular profile area and mean nuclear diameter of AVP cells in the dorsolateral supraoptic nucleus (SONdl) as a function of age. Bars indicate mean values per age group. Vertical lines indicate the SEM. n represents the number of brains examined. There is a significant effect of age on mean cellular profile (ANOVA, p<0.001), values in the 80-100-year group (*) being higher than those in the 20-60-year group (Student-Newman-Keuls; p<0.05). Open circles represent values in SDAT patients (Fliers et al., unpubl. observ.).
Figure 2. Basal levels of immunoreactive neurophysins from 20–100 years old. Note the similarity in the U-shaped curves of fig 1 and 2.

The suprachiasmatic nucleus (SCN) is considered to be the endogenous clock of the mammalian brain, which coordinates hormonal and behavioural circadian rhythms. Changes in rhythms and in particular in sleep have been reported in relation to ageing and senile dementia of the Alzheimer type (SDAT). Thus, the study of this nucleus in relation to age and SDAT is of particular interest. However, the very existence of this nucleus in the human brain was questioned until recently, simply because it was difficult to visualize it by means of the conventional histological staining techniques. Since immunocytochemical staining of this nucleus with antibodies against AVP appeared to be a good marker of the human SCN, similar to what had been found earlier in the rat, it became possible to apply morphological techniques to an investigation of the human SCN.

Methods

Brains of 31 patients, including 5 patients clinically diagnosed and pathologically confirmed as senile dementia of the Alzheimer type (SDAT), were obtained at autopsy. The brains were fixed in 10 per cent formaldehyde in distilled water generally for 1-2 months at room temperature. Following fixation the hypothalamic area containing the SCN, PVN and SON was dissected out, dehydrated in successive concentrations of ethanol, cleared in toluene, and embedded in paraffin. Serial 6 μm sections were cut transversely, mounted and brought to phosphate-buffered saline via xylene and graded ethanol series. For orientation, thionin staining (0.1 per cent thionin in acetate buffer, pH 4, 15 min) was used on every 50th section. From the central region of the PVN and SON, one section was selected for double immunoperoxidase staining and used for area measurements of AVP and OXT neurons in the SON and PVN.

The volume of the SCN was determined by staining every 25th section with anti-vasopressin, and at the rostral and caudal borders, every 5th section, followed by area measurements of the SCN. Subsequently, AVP cell density and, following thionin staining, the total cell density was measured. The number of AVP neurons and the total number of SCN cells were computed.

Results and discussion

The size and AVP cell profiles in the SONd1 (dorsolateral SON) and the PVN showed an initial decrease until the sixth decade, after which an increase was observed. (Fig 1). In brains from patients with SDAT, all morphometrical parameters were within the range of their respective control age group. Taking into account the relation between cell size and peptide synthesizing activity, the observed curve of AVP cell size may be interpreted in terms of activity of these cells. Therefore, our observations are in agreement with decreased levels of neurophysins between 50 and 60 years of age. In addition, an activation of AVP cells was found at older ages, which appears to be reflected in the peripheral levels of the peptide. Legros et al found a secondary increase of immunoreactive neurophysin blood levels after the age of 70 (Fig 2), which may be due to changes in kidney function. Furthermore, a similar U-shaped life-span pattern of hypothalamo-neurohypophysial system (HNS) activity was recently found in the rat. Consequently, the vasopressin "substitution therapy" in elderly and demented patients has probably been given to patients in whom the HNS was not deficient but activated.

A marked decrease in SCN volume, AVP cell number and total SCN cell number was found in 80-100 year-old patients as compared with the younger age groups (Table 1). Elderly people show substantial changes in sleep, i.e., more frequent awakenings, decreased REM sleep and reduced stage 4 sleep. These age-related changes seem to be more pronounced in men than in women. Similar changes in sleep variables, though greatly exceeding those observed in normal ageing, were found in demented patients, even in early, mild stages of SDAT. The decrease in SCN volume and cell numbers was also more pronounced in men than in women, and much more obvious in SDAT than in normal ageing (Table 1). The SCN changes in SDAT appeared earlier, and were stronger than those observed during normal aging.

From animal experiments it appears that the SCN cell number is important to its pacemaker properties. The decrease observed in SCN volume and cell number in senescence and, even more distinctly, in SDAT, suggests a causal role for the SCN changes in the sleep alterations. The proposed relationship between SCN changes and sleep changes during ageing, however, need further animal experimental confirmation.

In conclusion, it appears that the AVP neurons in the SON and PVN are activated in senescence, while SDAT patients follow this curve according to their age. In the SCN, on the other hand, a clear decrease
Table 1
Changes in the human suprachiasmatic nucleus with age and dementia (SDAT). Mean (± SEM).

<table>
<thead>
<tr>
<th></th>
<th>Group (years)</th>
<th>10–74</th>
<th>83–93</th>
<th>(SDAT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td></td>
<td>20</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td></td>
<td>42 (± 4)</td>
<td>88 (± 2)</td>
<td>77 (± 5)</td>
</tr>
<tr>
<td>Volume SCN (mm³)</td>
<td></td>
<td>0.276 (± 0.020)</td>
<td>0.187 (± 0.017)*</td>
<td>0.144 (± 0.040)*</td>
</tr>
<tr>
<td>Number of AVP cells</td>
<td></td>
<td>7.71 (± 0.58)</td>
<td>4.65 (± 0.37)*</td>
<td>3.48 (± 1.31)*</td>
</tr>
<tr>
<td>Total SCN cell number</td>
<td></td>
<td>50.98 (± 3.30)</td>
<td>34.94 (± 4.96)*</td>
<td>21.09 (± 6.70)*</td>
</tr>
<tr>
<td>Brain weight (g)</td>
<td></td>
<td>1389 (± 49.4)</td>
<td>1150 (63.4)*</td>
<td>1170 (± 135.1)</td>
</tr>
</tbody>
</table>

* = significant (p<0.03), by Mann-Whitney as compared to the young group (10–74 years of age).

in the amount of AVP neurons was observed in senescence, while a stronger and earlier decrease was found in SDAT. Since, depending on the area studied, the vasopressin neuron is either activated or degenerated, little specific benefit might be derived from the administration of exogenous vasopressin during ageing or dementia. Thus, our findings might explain the many negative results of clinical trials, involving vasopressin administration to these patient groups11.

Furthermore, our findings emphasize that SDAT can neither simply be seen as a ‘general degenerative process’ of the brain nor as a ‘cholinergic disease’.

Acknowledgements
This investigation was supported in part by the Foundation for Medical Research FUNGO (grant nr. 13-51-30). Reproduced with permission of Professor DL Knook (Eurage). Originally published in “Ageing of the brain and senile dementia: the inventory of EEC potentials,” DL Knook, G Calderini and L Amaducci (eds) pp 71-78, 1984.

References
Discussion

Dr Bleeker: I would like to ask Professor Swaab whether his findings on REM sleep reactivation in old rats could be extended to human beings.

Professor Swaab: I do not know yet.

Dr Bleeker: Do you know whether or not environmental stimuli change the activity of the brain in general?

Professor Swaab: There are encouraging indications that it might be possible to ameliorate the effects of dementia by adapting the environment to the needs of the patient (‘prosthetic environment’) and thus to improve the quality of life. Although the ability of the demented patients to learn and adapt remains reduced, worthwhile changes have been obtained. Some previous studies showed that social interactions could be improved by simple alterations in the pattern of ward activities or by introducing community activities. Bower, who implemented the idea that some symptoms of dementia were due to ‘sensory deprivation’, subjected the patients to ‘structured stimulation’. His findings were confirmed by Body, who reported an improvement in a number of behavioural functions. Various later studies gave similar results. The beneficial effects of some general measures (exercises, reactivation, adequate sleep and rest, occupational therapy etc) may be interpreted in the same spirit. A promising ‘environmental’ approach is the reality orientation therapy (ROT): Zepelin claimed that ROT over a one-year period prevented further mental deterioration in institutionalized psycho-geriatric patients.

Dr Ermini: The pineal traditionally is linked to circadian rhythms. Have you found any relationships between the various nuclei you were looking at and the pineal. What is known about changes in these structures?

Professor Swaab: No, so far we have only collected the pineals, and I can’t tell you anything about their activity in relation to age and dementia. By the way, they are often disregarded in neuropathology.

Professor Davison: Is there a more classical neurotransmitter associated with the vasopressin cell?

Professor Swaab: The brain is a network. So many of the classical transmitters are associated with the vasopressin cell. The key question at present is whether the acetyl choline system is connected to the vasopressin cell. As for the suprachiasmatic nucleus, I can only say that the cells of this nucleus are sensitive to acetyl choline and that it affects almost 100 per cent of the cells. So it seems probable that the suprachiasmatic nucleus is also innervated by acetylcholine. We do not have any histological evidence so far on this point, and I have to say that the antibodies we used in the human brain were not of a quality which made it possible to study this question. I do not know whether you can provide us with any antibody which is suitable for this type of study in the human brain.

Professor Bruyn: I would like to ask Professor Swaab whether he has any inkling of the main projection areas of the vasopressin cells in the human brain; I would like his answer to be that their main projection area is the temporal lobe, but I suspect that it won’t be.

Professor Swaab: In the rat brain, vasopressin cells do project to limbic structures. We are at present trying to trace the vasopressin fibres in the human brain in various age groups and in dementia. So far, the innervation of the temporal lobe does not appear impressive. The only structure that we found to be densely innervated with vasopressin fibres so far is the locus coeruleus.