

Neurofibrillary pathology in the infundibular nucleus in relation to age and abnormal hormone levels

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Abstract

Hyperphosphorylated-tau containing neurofibrillary (NF) Alzheimer-like pathology is present in the hypothalamic infundibular nucleus of cognitively intact control subjects. The NF changes show a striking sex difference: they are preferentially present in elderly men. We investigated the infundibular nucleus in postmortem material of young and old subjects with normal and abnormal hormone conditions, to determine whether the decline of testosterone in men during aging would make this nucleus more vulnerable for neurofibrillary (NF) changes (i.e. hyperphosphorylated tau) and, in women, whether the decline of estrogens in the postmenopausal period would protect the infundibular nucleus. Elderly male subjects with low testosterone conditions showed more severe NF changes in the infundibular nucleus than younger male controls and postmenopausal women. This NF pathology was not found in younger males or younger females with either normal or abnormal sex hormone levels due to hormone replacement therapy or hormone producing tumors. The occurrence of NF changes in aged subjects was generally accompanied by the presence of basket-like nerve terminals staining for ER β .

Our observations of these cases suggest that the sex difference in NF changes in the infundibular nucleus in elderly people is due to hyperphosphorylated-tau induction in a situation of gradual diminishment of testosterone during the process of aging in men, while in postmenopausal women a strong decline of estrogen levels seems to protect against NF changes in this brain area. Changes in circulating sex hormone levels alone in younger subjects are not sufficient to induce formation of NF changes in the infundibular nucleus.

KEY WORDS

Abnormal hormone conditions; Aging; Estrogen receptor α and β ; Human hypothalamus; Hyperphosphorylated-tau, Infundibular nucleus; Sex difference; Testosterone.

1. Introduction

The infundibular nucleus (= arcuate nucleus) of the hypothalamus is considered to be the central site of regulation of the hypothalamus-pituitary-gonadal (HPG) axis and of metabolism (1). Hyperphosphorylated tau-containing neurofibrillary (NF) pathology, which is observed in the infundibular nucleus of the hypothalamus of Alzheimer's disease patients, is also present in the hypothalamus of cognitively intact elderly subjects. This NF pathology shows a striking sex difference, i.e. it is almost exclusively present in the infundibular nucleus of cognitively intact elderly men and occurs only rarely in cognitively intact elderly women (2, 3).

In postmenopausal women a subset of neurons in the infundibular nucleus becomes strongly activated, as indicated histologically by an increased soma size, larger nuclei containing nuclear spheroids, larger and multiple nucleoli, and increased Nissl substance (4, 5). In addition, in postmenopausal women and in young subjects with a surgical menopause an increased gene expression was found in infundibular nucleus neurons for estrogen receptor (ER), neurokinin-B (NKB), substance-P (SP), and gonadotropin releasing hormone (GnRH) (4, 6, 7). A series of observations strongly suggests that the loss of inhibitory feedback of estrogens on the hypothalamus causes this increased neuronal hyperactivity in postmenopausal women (4). In addition, in elderly men some hypertrophied neurons were observed, but to a much lesser degree than in women (4, 8). In our previous study in the infundibular nucleus (5) a shift was observed from a more nuclear localization of ER α in young females to a more cytoplasmic localization of ER α in non-demented postmenopausal women. This shift in ER α localization was accompanied by a relative absence in the expression of NF pathology, i.e. hyperphosphorylated-tau stained by AT8 in the infundibular nucleus, and was considered to be another sign of neuronal activation. We therefore considered the sex difference in NF pathology as one of the many examples of neurons that

were highly active or strongly activated in the elderly and seemed to be protected against the occurrence of NF pathology, a phenomenon we described as “use it or lose it” (9, 10).

In non-demented elderly men only a small increase in cytoplasmic ER α was found, accompanied by NF pathology in the infundibular nucleus. In addition, the occurrence of NF pathology in non-demented elderly men was accompanied by the presence of more ER β -containing basket-like nerve terminals in the infundibular nucleus (5), and both subtypes of ERs were therefore studied with the present materials.

In order to determine whether the gradual diminishment of testosterone in men during aging would induce NF pathology or whether the strong decline of estrogens in postmenopausal women would protect the infundibular nucleus neurons against NF pathology, we investigated the infundibular nucleus and adjacent areas in postmortem material of patients with abnormal hormone conditions as judged by their clinical data and by staining of the androgen receptor (AR) in the medial mamillary nucleus (MMN), which is the area of the hypothalamus most sensitive to changes in circulating androgen levels (16).

2. Materials and Methods

2.1. Tissue collection

Postmortem material was obtained from the Netherlands Brain Bank (coordinator Dr. R. Ravid). Permission was obtained for a brain autopsy and for the use of brain material and clinical information for research purposes. Hypothalami of 13 subjects with abnormal circulating gonadal steroid levels were studied immunocytochemically. These levels were: i.e. castrated, estrogen-treated male-to-female (MF) transsexuals; an ovariectomized testosterone-treated female-to-male (FM) transsexual; castrated prostate cancer patients; an ovariectomized woman; a subject with complete androgen insensitivity syndrome (CAIS); a subject with an estrogen-producing adrenal tumor, a subject with an androgen-producing

adrenal tumor (table 2), and 16 age and sex-matched control subjects (table 1). None of the subjects suffered from a primary neurological or psychiatric disease. All the brains were investigated in a systematic way by a neuropathologist (for details see (11)). The distribution of the Alzheimer neurofibrillary changes over the brain were estimated according to the stages of Braak and Braak (12, 13). Six stages of disease propagation can be distinguished with respect to the location of intraneuronal cytoskeletal changes, i.e. neuropil threads and neurofibrillary tangles stained with Bodian silver staining, and with respect to the severity of changes in the hippocampal formation, in the transentorhinal and entorhinal regions and in the adjoining temporal isocortex (12). In Braak stage 0 no NF pathology is observed. Braak stages I-II, clinically silent cases, are characterized by the presence of neurofibrillary changes confined to the transentorhinal region. Braak stages III-IV, incipient AD, are characterized by severe changes found only in a few allocortical regions and adjoining areas. Stage III reveals the striking destruction of layer Pre- α within both the entorhinal and transentorhinal regions, and is accompanied by mild changes in the hippocampus and by the virtual absence of neocortical lesions. In stage IV, additional changes are found in the deep layer of Pre- α . Braak stages V-VI, fully developed in Alzheimer's disease, are characterized by the destruction of neocortical association areas (13).

The hypothalami were formalin-fixed, paraffin-embedded, and cut serially in 6 μ m coronal sections. For anatomical orientation, every 100th section was mounted on chrome-alumsulphate-coated glass slides, deparaffinized, hydrated, and stained with thionine (0.1% w/v thionine in acetate buffer, pH 4). The location of the infundibular nucleus was determined based on the human brain atlas according to Mai et al (14), and if necessary with the help of neuropeptide Y (NPY) immunocytochemical staining (1, 15). The rostral border of the infundibular nucleus was identified at the level where the nucleus showed its characteristic arcuate shape, with the cell-sparse zone separating the infundibular nucleus from the

ventromedial nucleus (VMN), indicating the dorso-lateral border, the ependymal layer of the third ventricle serving as the medial border and the mamillary bodies taken as the caudal border. Three series of sections per subject were taken from rostral to caudal at approximately 25%, 50% and 75% of the length of the infundibular nucleus, and mounted onto Super-Frost plus (Menzel, Braunschweig, Germany) slides for immunocytochemistry. We took adjacent sections for estrogen receptor (ER) α , ER β and hyperphosphorylated-tau protein (AT8) immunocytochemical staining. In addition, we also determined the expression of AT8 staining in other areas in the hypothalamus adjacent to the infundibular nucleus, such as the VMN, the nucleus tuberalis lateralis (NTL), the nucleus basalis of Meynert (NBM), and the tuberomamillary nucleus (TMN). Since the serum levels of testosterone in subjects from which we had autopsy material was unavailable, we determined the expression of nuclear AR-ir in the MMN as an indirect measure of circulating androgen levels (16).

2.2. Immunocytochemistry and specificity of the antisera

A polyclonal rabbit anti-ER α antiserum (MC-20) that recognizes the carboxyl-terminus epitope of the ER α (SantaCruz Biotechnology, Inc., catalogue no. sc-542) and a polyclonal goat anti-ER β antiserum (N-19), directed against an amino acid sequence mapping at the amino-terminus of human ER β (catalogue no. sc-6820, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) were used in the present study. The staining procedures and specificity tests for ER α and ER β antisera have been described extensively by Ishunina et al (17) and Kruijver et al (18, 19). No staining was observed after omitting the MC-20 antiserum or after adsorption of MC-20 to its blocking peptide (17). In a spot blot test, MC-20 recognized its blocking peptide on nitrocellulose paper by showing the expected concentration gradient (Santa Cruz Biotechnology; blocking peptide, catalog no. sc-542, lot no.C059). In addition, two different anti-ER α antisera, C-314 (N-terminus directed; Santa Cruz Biotechnology; Catalog no. sc-786;

anti-bovine ER α ; lot no.J278) and MC-20 (C-terminus directed) displayed similar distribution patterns in the human hypothalamus (18). Western blot with the ER α antiserum MC-20 on human hypothalamic tissue showed a specific band around the expected 68 kDa, with no such band around the 54 kDa of ER β (18, 20, 21). Western blot with the ER β antiserum N-19 on human hypothalamic tissue recognized a protein band around the expected 54 kDa weight and did not recognize the 68 kDa protein band, i.e. ER α (19, 20). In spot blots it was also confirmed that the antiserum N-19 recognizes the homologous blocking peptides, while an adsorption test with the homologous peptide resulted in elimination of the staining (17). Moreover, staining of adjacent sections with the antiserum against the C-terminus of the ER β (L-20, Santa Cruz Biotechnology, Inc, catalogue no. sc-6822)(22) revealed the same staining pattern as the antiserum against the N-terminus of ER β used in the present study (17). ER β cytoplasmic staining was observed in granulosa cells and follicles of the human ovary, a localization which is consistent with a study in the rat (23). In human testis, Leydig and connective tissue cells showed nuclear ER β staining, which is also in agreement with a study in rat (24). The differences in distribution shown by the ER α antiserum MC-20 and the ER β antiserum N-19 in the hypothalamus, pituitary, ovary and testis, as described extensively by Ishunina et al (17) and Kruijver et al (18, 19), supported the specificity of the antisera. This series of observations demonstrated that the ER α and ER β antisera used in our study were specific.

For immunocytochemical staining of hyperphosphorylated-tau, a primary monoclonal antiserum AT8 directed against the phosphorylated-tau epitopes serine 202 and threonine 205 (25, 26) was used. This antiserum was used to recognize hyperphosphorylated-tau as an early marker for neurofibrillary AD pathology. The staining procedure was performed according to Schultz et al (2).

Earlier studies showed that the variability in fixation and postmortem time does not influence

the staining of ER α , ER β or AT8 (5, 18, 19).

Immunocytochemical staining for AR in the MMN was based on the protocol described in Kruijver et al. (16), using a different primary anti-human AR antibody, i.e. ARIE (batch nr# 040898) raised in our institute against the first 20 amino acids of human AR peptide sequence of the C-terminus, coupled to thyroglobulin by glutaraldehyde. For details of the procedure and specificity of this antibody see (Bao et al., 2006).

2.3. Analysis of the staining intensity

Two independent investigators, blind to the subject's condition, judged the staining intensity of the sections. The staining intensity of ER α and ER β in the cytoplasmic and nuclear compartment was estimated semi-quantitatively by means of light microscopy, based on the number of stained neuron and basket-like nerve terminals in the infundibular nucleus, and graded according to the following scale: (+++) strong, (++) moderate, (+) weak, (+/-) very weak and (-) absent, according to our previous studies (17). The semi-quantitative estimation for neuropathological changes in AT8-staining was judged according to the number of stained neurons and neuropil threads in the infundibular nucleus and graded by Schultz et al. (2) on the following scale: (+++++) severe, (++++ marked, (++) moderate, (+) mild, (0) no discernible changes.

In addition, the semi-quantitative estimation for AR staining in the MMN was judged based on the following scale: (+++) strong, (++) moderate, (+) weak, (+/-) very weak and (-) absent.

3. Results

Subjects who were assumed to have low androgen levels showed lower or absent expression of nuclear AR in the MMN (Table 2).

3.1. Control subjects (table 1, figs. 1, 2)

Female controls (Braak stage 0-II) did not show NF pathology as stained by AT8 in the infundibular nucleus, while mild changes were only observed in the 2 oldest males (#15, #16, age 78 and 80, respectively). Staining of hyperphosphorylated-tau was absent in other hypothalamic or adjacent brain areas in controls.

Histological signs of activation, i.e. hypertrophied neurons and nuclear spheroid-containing neurons, were clearly present in two elderly female controls (#6, #7), while fewer of such changes were observed in another elderly female (#8) and in the 2 oldest male controls (#15, #16; fig. 2). Using adjacent sections, we observed that the hypertrophied neurons in the 2 oldest male controls (#15, #16) did not show NF pathology.

Cytoplasmic ER α was not present in the youngest 2 females, while nuclear ER α was clearly stained. From age 49 onwards cytoplasmic ER α was generally more strongly stained in the infundibular nucleus of female controls than in male controls, while more nuclear ER α was observed in male controls than in female controls. Only weak staining of both nuclear and cytoplasmic ER β was observed in the infundibular nucleus of male and female controls (fig. 1). Basket-like ER β -staining was observed more in males than in females.

In the MMN more nuclear AR was observed in male controls than in female controls (table 1).

3.2. Subjects with abnormal hormone conditions (table 2, figs. 1,2)

All 5 subjects that underwent castration because of prostate cancer showed AT8 positive staining in the infundibular nucleus, independent of whether they were treated or not treated with anti-androgens. All 5 patients in this group showed more severe NF pathology than observed in male controls of comparable ages (fig. 2). Two of these patients (#17, #21) showed also hyperphosphorylated-tau in other hypothalamic and adjacent brain areas, i.e. the ventromedial nucleus (VMN), the nucleus tuberalis lateralis (NTL), the nucleus basalis of

Meynert (NBM) and the tuberomamillary nucleus (TMN) (table 2).

The subject with complete androgen insensitivity syndrome (CAIS) (#22), who had received estrogen substitution until 2 months before death, did not show NF pathology in the infundibular nucleus.

The 74-year-old male-to-female (MF) castrated and estrogen-treated transsexual (#25) had moderate AT8-staining in the infundibular nucleus, whereas in two younger MF transsexuals (#23, #24) the presence of hyperphosphorylated-tau was not observed. A female- to-male (FM) transsexual subject (#27) of 51 years of age who had been treated with testosterone showed no AT8-staining. In the 46-year-old ovariectomized female (#26), in a 46-year-old female with an androgen-producing adrenal tumor (#28) and in a 31-year-old male with an estrogen-producing adrenal tumor (#29) no AT8-staining in the infundibular nucleus was observed either.

An absent to weak staining of nuclear ER α and a weak to moderate staining of cytoplasmic ER α were observed in the infundibular nucleus of prostate cancer patients, MF transsexual subjects, ovariectomized woman and a FM transsexual. A moderate nuclear ER α and a very weak to moderate cytoplasmic ER α -staining were observed in the CAIS subject and in subjects with sex steroid-producing adrenocortical carcinoma. Concluding, no clear alteration in relation to the abnormal hormone levels was observed.

All subjects with abnormal hormone conditions showed an absent to very weak nuclear ER β -staining and a very weak to weak cytoplasmic ER β -staining. Concluding, the nuclear and cytoplasmic ER β staining did not reveal any specific information.

Subjects with low levels of testosterone showed relatively more basket-like ER β than elderly control subjects (fig. 1). The occurrence of hyperphosphorylated-tau in a subset of neurons in the infundibular nucleus of elderly males in low testosterone conditions was generally accompanied by the presence of basket-like ER β in nerve terminals (#18, #19, #20, and #25).

Hypertrophied neurons were observed more in elderly castrated prostate cancer patients than in other subjects with abnormal hormone conditions. These neurons showed histological signs of hyperactivity, i.e. larger cell size, a larger nucleus and nucleolus compared to the surrounding neurons and the presence of nuclear spheroid bodies. They were observed easily, either located inside or outside basket-like ER β nerve terminals. Remarkably, these hyperactive neurons never showed NF pathology.

4. Discussion

The infundibular nucleus, a key structure in the regulation of reproduction and metabolism (1) shows remarkable neurofibrillary (NF) Alzheimer (AD)-like changes in cognitively intact subjects (with Braak stage 0-II). The NF pathology in the infundibular nucleus is characterized by neurofibrillary tangles, a network of neuropil threads and terminal-like portal vessel-associated processes. This NF pathology shows a striking sex difference. From 60 years onwards the prevalence of neurofibrillary changes in the infundibular nucleus of cognitively intact elderly males rises from 20% up to 90% around the age of 80-85 years, while in only 6-10% of cognitively intact elderly females such changes were observed (2, 3, 5, 27, 28). Our present control data confirms these previous findings. Moreover, we showed for the first time that changes of circulating sex hormone levels alone are not sufficient to induce formation of NF changes in the infundibular nucleus, while decreasing testosterone levels during aging stimulate the formation of NF changes.

Circulating total testosterone in men derives both from testicular Leydig cell secretion and from peripheral conversion of dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEA-S), that is produced by the adrenals (29). Approximately 90% of the serum testosterone in men originates from the testes, whereas only 67% of the serum testosterone in women is secreted by the ovaries (30). Changes of sex hormones during aging remain a

controversial topic in literature. The reference data from the Endocrinology Department of the Academic Medical Center Amsterdam, The Netherlands (Dr. E. Endert) shows that men have quite stable though slowly decreasing testosterone levels (from 22 nmol/l age 21~30 to 17 nmol/l age 60~70) and stable estradiol levels (around 0.14 nmol/l up to age ~70), while women have much lower slightly decreasing testosterone levels (from 3nmol/l age 21~30 to 1.25 nmol/l age 61~70). Their estrogen levels decline abruptly after age 50 (menopause), i.e., estradiol level declines from 0.27 to 0.02 nmol/l. These data fit in well with our present findings that nuclear AR staining in the MMN is generally higher in men than in women and decreases only after age 70 in men. This also confirms that AR staining in the MMN can be used as a monitor for circulating testosterone levels (16).

In postmenopausal women, the abrupt decline in circulating estrogen levels (31) and the diminished negative feedback of estrogens on the infundibular nucleus neurons (32-34) is accompanied by a strong activation of neurons in this brain area, as shown by histological signs of neuronal hypertrophy and increased amounts of estrogen receptors (ER), neurokinin B (NKB) or substance P (SP) gene transcripts in this nucleus (4, 6, 35). In a previous study, the shift from a more nuclear localization of ER α in young females to more cytoplasmic ER α in the infundibular nucleus neurons in cognitively intact postmenopausal women was found to be accompanied by other signs of neuronal hyperactivity and by a lower vulnerability of these neurons to develop NF pathology as compared to males (5). In addition, taking into consideration the relatively young patients with abnormal hormone levels, i.e. the surgical menopausal case (#26, age 46, ovariectomized without hormone replacement), the androgen-producing tumor case (#28, age 46), and the FM transsexual case (#27, age 51, castrated with androgen replacement but stopped 3 years before death), all of which lacked NF pathology, we conclude that changes of sex hormone levels alone are not sufficient to induce NF pathology in the infundibular nucleus of young females, while in postmenopausal

women the decrease of estrogen levels seems to protect against the occurrence of age-related NF changes in this brain area (table 1).

In elderly men the reported gradually decreasing plasma testosterone levels (36) are accompanied by a lesser degree of neuronal hypertrophy than observed in women (8). It is therefore of interest to find in the present study that mild NF pathology in the infundibular nucleus in controls was only observed in the 2 oldest males (#15, #16, age 78 and 80, respectively) while all of the 5 old castrated prostate cancer patients showed more NF pathology than male controls of comparable ages, independent of the fact whether subjects did (#18, #21) or did not (#17, #19, #20) receive anti-androgen treatment following orchidectomy (tables 2,3). Hyperphosphorylated-tau as stained by AT8 was also observed in other brain areas in elderly castrated prostate cancer patients, such as in the ventromedial nucleus (VMN), the nucleus tuberalis lateralis (NTL), the nucleus basalis of Meynert (NBM) and the tuberomamillary nucleus (TMN) (#17, #21) and was accompanied in one patient (#20) by subcortical dementia symptoms, suggesting that low testosterone levels may also be involved in the development of NF pathology in cognition-related areas in the hypothalamus, i.e. the NBM and TMN. The observation that the decline of testosterone in old males especially after the age of 70 and in old castrated men is accompanied by a mild to moderate NF pathology in the infundibular nucleus, means that androgens seem to protect against NF changes formation in the infundibular nucleus in males. In women, on the contrary, testosterone does not change significantly in relation to the menopausal transition (37). A significant fall in circulating levels of sex steroid hormone-binding globulin (SHBG) across menopausal transition may result an increase in free androgen levels, as indicated for example by an increase in free androgen index (FAI) (37). The idea of remain constant or slightly decrease of testosterone levels across menopausal years (38-40) is in agreement with our study which observed a relatively constant of AR expression in the MMN in women with

different age (table 1.) We are tempting to suggest if this observation might be related to protective action of testosterone for NF pathology formation in women since women in general hardly ever show age related-NF pathology in the infundibular nucleus.

We observed a lack of NF pathology in the case of the young male with an estrogen-producing tumor (#29, age 31, with both higher testosterone and estrogen levels) and in the two rather young MF transsexuals (#23 and #24, age 50 and 53, with lower androgen levels and higher estrogen levels, respectively). In addition, no NF pathology was found in the CAIS patient (#22, age 75) who was phenotypically a heterosexual woman and had higher estrogen levels during her life until the last two months before her death at age 75. It should be noted that NF staining in control males starts in the seventies (table 1, ref. 15). The presence of NF pathology in the infundibular nucleus of a 74-year-old MF transsexual who had lower androgen levels and higher estrogen levels (#25) may be based on the variability of the occurrence of this pathology in the seventies. These findings implicated that the process of aging is another requirement for the occurrence of NF pathology. In other words, the decline of androgen levels in men during aging is only one of the factors involved in the formation of AD-like pathology.

In a previous study we found that a subset of proopiomelanocortin (POMC) neurons in the infundibular nucleus co-expresses hyperphosphorylated-tau as stained by AT8, both in cognitively intact elderly males and in AD patients (5). In rodent, the cellular POMC mRNA content in the arcuate nucleus was significantly lower in old males than in young males (41). Moreover, the abruptly decreased testosterone levels following castration of adult male rhesus monkey results in a strong suppression of the mRNA production of POMC neurons (42). This indicates that indeed both aging and low testosterone conditions may contribute to reduced metabolic activity of POMC neurons in the hypothalamus and so increase the risk of these neurons to develop NF pathology in males. In our present study, low testosterone conditions

also seemed to increase the risk for elderly males to get NF pathology in areas which are related to memory and attention (i.e. the NBM and TMN) (#17, #21), which suggests that such a mechanism may also take place in other parts of the brain.

In a previous study we observed the occurrence of basket-like nerve terminals staining for ER β in the infundibular nucleus in two conditions: (i) in elderly men and (ii) in AD patients in relation to the occurrence of NF pathology in this nucleus (5). In the present study, the occurrence of hyperphosphorylated-tau in elderly hypogonadal men was generally also found to be accompanied by the presence of basket-like nerve terminals staining for ER β in the infundibular nucleus (table 2). Our ongoing study showed that such basket-like ER β -containing terminals co-express glutamic-acid decarboxylase (GAD) immunoreactivity as a marker for the inhibitory transmitter γ -amino butyric acid (GABA) (43). The neurons inside the ER β staining basket tended to be larger and to have a larger nucleus and nucleolus than the surrounding neurons, which suggests that the neuron inside the basket is strongly activated while these neurons remain free of NF changes in the middle of NF neuropathology that is derived from the subpopulation of POMC neurons. We also observed this phenomenon of intact, activated neurons surrounded by a basket like terminal and free of NF pathology in AD patients (5). That the presence of ER β basket and NF changes do not always go together makes it clear that they are in principle two independent phenomena. We hypothesized, therefore, that ER β -mediated inhibition of the GABAergic terminals may induce an increased activity of the neurons inside the basket and thus prevent the formation of hyperphosphorylated-tau in this subpopulation of neurons, which is in line with the phenomenon we described as “use it or lose it” (9, 10).

Concluding, our observations suggest that the sex difference in NF changes in the infundibular nucleus in elderly people is due to hyperphosphorylated-tau induction in a situation of gradual diminishment of testosterone during aging in men, as is clear following

castration. The NF changes occur in a subpopulation of neurons containing POMC. However, the process of aging seems to play a more important role in the formation of AD-like pathology than the changes in sex hormones. In postmenopausal women the strong decline of estrogen levels seems to protect against NF changes in this brain area. Changes in sex hormone levels appeared to be only one of the many factors involved in the etiology of AD changes in the infundibular nucleus. However, since testosterone was found to be of importance and since substitution may prevent the formation of heat shock-induced hyperphosphorylation of tau in rats (44), further investigation of the efficacy of testosterone substitution therapy for delaying or preventing the occurrence of NF pathology in the brain of elderly males seems worthwhile. A second phenomenon was the increased activity of some neurons in the infundibular nucleus of hypogonadal elderly males, probably mediated by surrounding basket-like nerve terminals containing ER β , accompanied by an absence of neurofibrillary pathology, even in the middle of NF changes in this nucleus. This phenomenon, which is also present in postmenopausal women (5) indicates a new mechanism in the local prevention of NF pathology.

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Figure legends:

Figure 1. Photomicrograph depicting basket-like nerve terminals containing estrogen receptor (ER) β immunoreactivity (IR) in the infundibular nucleus. Hypogonadal prostate cancer patients (subject #20)(B) showed more of such baskets with a higher intensity of ER β -IR than elderly male controls (subject #16)(A). Scale bar: 20 μ m.

Figure 2. Photomicrograph depicting hyperphosphorylated-tau stained by AT8 in the infundibular nucleus. Hypogonadal prostate cancer patients showed more severe AT8 positive staining (subject #20)(B) than elderly male controls (subject #16)(A). Scale bar: 20 μ m.

Figure 3. This figure shows the correlation between expression of nuclear androgen receptor (AR) staining in the MMN and assumed circulating testosterone levels.

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Figure 1.

Figure 2.

Figure 3.

Table 1. AT8, ER α and ER β staining in the infundibular nucleus of control subjects

No	NBB	Age	Sex	Bw(g)	Pmd(h)	Fix(d)	BS	AT8	ER α		ER β			AR
									(INF)		(INF)		(MMN)	
									N	C	N	C	B	N
1	86032	33	F	1035	41.00	20	0	-	2+	-	+	\pm	+	2+
2	80008	35	F	1200	08.00	26	0	-	3+	-	-	\pm	\pm	-
3	96423	49	F	1253	<17.00	806	0	-	\pm	2+	2+	+	-	2+
4	98125	58	F	991	06.15	41	I	-	+	2+	-	\pm	-	-
5	98035	65	F	ID	<20.00	31	0	-	+	+	-	+	-	+
6	99085	69	F	1102	<02.30	120	0	-	\pm	2+	-	+	-	2+
7	93139	78	F	1135	06.25	32	0	-	-	2+	\pm	+	+	+
8	96084	78	F	1330	07.30	26	II	-	2+	+	-	\pm	-	+
Median value								-	+	2+	-	\pm	-	+

9	98121	47	M	1420	<82.30	31	0	-	±	±	-	+	-	2+
10	97159	48	M	1500	05.30	42	0	-	2+	±	-	±	2+	2+
11	93072	50	M	1573	<09.00	52	0	-	+	+	-	+	+	2+
12	97139	59	M	1400	<65.45	180	0	-	±	+	-	±	-	2+
13	98122	66	M	1461	<41.00	49	0	-	2+	+	-	+	+	2+
14	96426	69	M	1222	14.00	728	0	-	2+	2+	±	±	-	3+
15	94076	78	M	1442	08.25	24	II	+	2+	+	-	±	2+	-
16	97116	80	M	1380	06.56	33	0	+	3+	+	±	±	-	-
Median value								-	2+	+	-	±	±	2+

Abbreviations: AdenoCa: adenocarcinoma, AR: androgen receptor, AT8: specific staining for hyperphosphorylated-tau, B: basket-like, BS: Braak score, Bw: brain weight (in grams), C: cytoplasmic staining, ER: estrogen receptor, F: female, Fix: fixation time (in days), ID: incomplete data, INF: the infundibular nucleus, N: nuclear staining, NBB: Netherlands Brain Bank number, ND:

can not be determined, M: male, MMN; medial mammillary nucleus, Pmd: postmortem delay (in hours). Note that AT8 staining in the infundibular nucleus is only present in the 2 oldest males.

Table 2. AT8, ER α and ER β staining in the infundibular nucleus and other brain areas of subjects with abnormal hormone conditions

No	NBB	Diagnosis	Age	Sex	Bw(g)	Pmd(h)	Fix(d)	BS	AT8 (INF)	AT8	ER α		ER β		AR	
										in	(INF)	(INF)	(MMN)			
										TMN,	N	C	N	C	B	N
										VMN,						
										NBM						
17	89103	Prostate cancer	67	M	1290	24:00	28	ID	2+	NTL(2+), TMN(2+), VMN(2+), NBM(2+)	-	+	-	\pm	\pm	+
18	97157	Prostate cancer	69	M	1475	05:55	45	0	4+	-	\pm	+	\pm	+	2+	-
19	95062	Prostate cancer	80	M	1400	04:30	24	II	4+	-	\pm	+	-	\pm	2+	-
20	94109	Prostate cancer	82	M	1110	05:35	32	II	4+	-	\pm	+	-	\pm	3+	-

21	94090	Prostate cancer	86	M	1663	03:00	93	II	3+	TMN(+), VMN(+), NBM(2+)	+	2+	-	±	+	±
22	02089	CAIS	75	M	1484	06:30	34	I	-	-	2+	2+	-	±	-	-
23	84020	MF Transsexual	50	M	1380	ID	30	0	-	-	±	-	-	±	±	±
24	93070	MF Transsexual	53	M	1500	<100	34	0	-	-	-	±	-	±	±	+
25	98141	MF Transsexual	74	M	1118	06:35	33	I	2+	-	±	2+	-	+	2+	-
26	80002	Surgical menopause	46	F	1300	02:30	36	0	-	-	-	+	-	±	-	+
27	98138	FM Transsexual	51	F	1171	04:15	32	0	-	-	+	+	-	+	±	2+
28	83004	Androgen-producing adrenal tumor	46	F	1360	<10:50	34	ID	-	-	2+	±	-	±	+	2+
29	91005	Estrogen-producing adrenal tumor	31	M	1377	<34:00	35	ID	-	-	2+	+	-	±	2+	2+

Abbreviations: AR: androgen receptor, AT8: staining for hyperphosphorylated-tau, B: basket-like, BS: Braak score, Bw: brain weight

(in grams), CAIS: complete androgen insensitivity syndrome, C: cytoplasmic staining, ER: estrogen receptor, F: female, FM: female-to-male transsexual, Fix: fixation time (in days), ID: incomplete data, INF: the infundibular nucleus, N: nuclear staining, NBB: Netherlands Brain Bank number, M: male, MF: male to female transsexual, MMN; medial mammillary nucleus, NBM: the nucleus basalis of Meynert, ND: cannot be determined, NTL: the nucleus tuberalis lateralis, ORX: orchidectomy, OVX: ovariectomy, Pmd: postmortem delay (in hours), TMN: the tuberomammillary nucleus, VMN: the ventromedial nucleus.

Table 3. List of control subjects and the cause of death

No	NBB	Age	Sex	Cause of death
1	86032	33	F	AdenoCa metastases to brain
2	80008	35	F	Acute lymphoblastic leukemia
3	96423	49	F	Massive thromboembolism
4	98125	58	F	Multiple organ failure
5	98035	65	F	Mesenterical ischemia
6	99085	69	F	Uremia
7	93139	78	F	Bronchopneumonia
8	96084	78	F	Pulmonary emphysema
9	98121	47	M	Cardiac arrest
10	97159	48	M	Multiple organ failure
11	93072	50	M	Hypovolemic shock

12	97139	59	M	Pulmonary embolism
13	98122	66	M	Septic shock
14	96426	69	M	Septic shock
15	97116	80	M	Pulmonary emphysema
16	94076	78	M	Myocardial infarction

Abbreviations: F: female, M: Male, NBB: Netherlands Brain Bank number

Table 4. Clinical and endocrine history of patients with abnormal hormone conditions

Case	No.	Age (yr)	Age of hormonal treatment (yr)	Age of Orchid-/Ovari-ectomy (yr)	Clinical endocrine history	data and Cause of death	Assumption androgen status as compared to controls	Assumption estrogen status as compared to controls
Prostate cancer patients	#17	67	-	67	Orchidectomy 3 months before death, patient did not receive anti-androgen treatment	Carcinoma of pancreas with metastases; cachexia	↓	↓

				Orchidectomy 3 years		
				before death, Prostate cancer		
#18	69	67	67	anti-androgen, anandron with advanced	↓	↓
				150mg 1 dd during 3 metastases		
				years before death		
				Orchidectomy 5 years		
#19	80	-	75	before death, patient did Renal	↓	↓
				not receive anti-androgen insufficiency		
				treatment		
				Orchidectomy 20 years Prostate cancer,		
#20	82	-	82	before death, patient did respiratory	↓	↓
				not receive anti-androgen failure, renal		
				treatment insufficiency		

#21 86 85 85

Memory problem started

6 years before death.

Diagnosis at

hospitalization:

subcortical dementia.

Neuropathological

diagnosis: slight

alzheimerization, Braak

for tangle = II.

Orchidectomy 1 year

before death. Patient

received CPA,

anti-androgen, (50mg 4

dd) during the first 14

months, (50 mg 2 dd)

during the last 6 months

Prostate cancer,

lung cancer and ↓

septic shock ↓

					Orchidectomy 20 years		
					before death, patient		
					received 17β-estradiol	Advanced state	
CAIS	#22	75	70	55	(2mg 1 dd) during the	of squamose cell ↓	↑
					last 5 years before death,	vagina carcinoma	
					and stopped 2 months		
					before death.		

					Age 42: stilbestrol (5 mg			
					1 dd); after 2 months to			
					(5 mg 2 dd); age 44: CPA			
					(50 mg 2 dd); treatment			
MF-transsexuals	#23	50	42	44	lasted 4 years; stopped 2	Suicide	↓	↑
					years before death;			
					ethinylestradiol (50 µg 2			
					dd); treatment lasted 8			
					years until death			

#24	53	40	50	<p>Age 40: stilbestrol treatment (stopped after 1 yr); at age 43–47: premarin (0.625 mg dd); at age 47–50: Premarin (3.75 mg dd); at age 50–53: Premarin (2.5 mg 3 dd); CPA (50 mg 1 dd); topical estrogen cream (estrogen treatment stopped 3 months before death)</p>	<p>Acute fatty liver due to alcohol ↓ abuse</p>	↑
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					Age 64: received CPA treatment (50 mg 2 dd) and ethinyl estradiol (50 µg 2 dd) treatment; at age 67: received estraderm (100 µg 1 dd); at age 74 received spironolactone (50 mg 1 dd) and estraderm (100 µg 1 dd)	Coma post-appendicitis, pneumonia, lung embolism, and occipital cerebral infarction	↓	↑
Surgical Menopause	#26	46	-	45	Bilateral ovariectomy 22 months before death, patient did not receive hormone treatment	Septicemia and ovarian cancer	↓	↓
FM-transsexual	#27	51	27	32	Bilateral ovariectomy at age 32. At age 27	Cachexia	↑	↓

testosterone, Sustanon
(250 mg), twice a month
injections; at age 30
testosterone undecanoate
(40 mg 3 dd); at age 34
testosterone undecanoate
(40 mg 2 dd); at age 36
testosterone undecanoate
(40 mg 4 dd); at age 44
testosterone, Sustanon
(250 mg) twice a month
injections; at age 47–48
testosterone, Sustanon
(250 mg) every 3 weeks.
No testosterone

					replacement therapy	
					during the last 3 years	
					before death	
					Female patient with a	
					virilizing adrenocortical	
					carcinoma for 1 yr that	
					produced high levels of	
					cortisol, androstenedione, Adrenocortical	
Androgen-producing	#28	46	-	-	and testosterone levels; carcinoma;	↑
adrenal tumor					latest androstenedione postoperative	(*)
					serum level before death hemorrhage	(measured)
					was 48.0 ng/ml (normal	
					range for women 0.4–3.5	
					ng/ml); the latest serum	
					testosterone level before	

				death, 26.82 nmol/L (normal range for women is 1.04–3.30 nmol/L).	
Estrogen-producing adrenal tumor	#29	31	-	-	<p>Male patient with the recurrent feminizing adrenocortical carcinoma for 3 yr that produced high levels of DHEA-S, DHEA, 17-hydroxyprogesterone, and estradiol levels; the latest estradiol serum levels 1 yr before death was around 689-732 pmol/L (normal range for</p> <p>Advanced metastasis of recurrent adrenocortical carcinoma</p> <p>Slightly ↑ (measured)</p> <p>↑ (measured)</p>

men is 50-200 pmol/L);
the latest testosterone
levels 1 year before death
was around 28.9-41.3
nmol/L (normal range for
men is 10-30 nmol/L)

Abbreviations: CAIS: complete androgen insensitivity syndrome, CPA: cyproterone acetate, DHEA: dehydroepiandrosterone, DHEA-S: dehydroepiandrosterone-sulphate, No: numbers corresponding with table 2, (*): cannot be assumed.