Research Reports

Changes with Aging in the Vasopressin and Oxytocin Innervation of the Rat Brain

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The effect of aging on the vasopressin (AVP) and oxytocin (OXT) innervation of the brain was studied by means of immunocytochemistry, comparing the major innervated areas in 5-month-old and 34-month-old male Brown-Norway rats. A marked decrease of AVP fiber density was found in the old rats as compared with the young animals in the vertical limb of the diagonal band, the basal nucleus of Meynert, the lateral habenular nucleus, the medial amygdaloid nucleus, the substantia nigra, the ventral hippocampus, the central gray, the locus coeruleus and in the ambiguous nucleus. The AVP innervation of the lateral septum and the dorsomedial hypothalamic nucleus was moderately, although not significantly reduced. No age difference in AVP innervation was found in the paraventricular thalamic nucleus or in the nucleus of the solitary tract. OXT fiber density did not differ between young and old animals in the locus coeruleus, the nucleus of the solitary tract and the ambiguous nucleus. Thus, the aging process appears to affect AVP cells in a differential, rather than in a general way. Changes were found to be more pronounced in those areas where the AVP innervation is dependent upon circulating androgens.

INTRODUCTION

Immunocytochemical studies have demonstrated that apart from the paraventricular (PVN), supraoptic (SON) and suprachiasmatic nucleus (SCN)^{31,35}, vasopressin (AVP) cells are present in the bed nucleus of the stria terminalis (BST), the medial amygdaloid nucleus, the dorsomedial hypothalamic nucleus and the locus coeruleus^{4,37}. By contrast, oxytocin (OXT) cells were found exclusively in the SON and PVN. By tracing and lesion studies, it was demonstrated that AVP cells in the PVN project to the nucleus ambiguus, the nucleus of the solitary tract and the spinal cord^{8,28}, while the AVP cells in the SCN project to the dorsomedial hypothalamic nucleus, the vascular organ of the lamina terminalis and the paraventricular thalamic nucleus¹⁹. AVP fibers in the diagonal band of Broca, the lateral septum, the lateral habenular nucleus and locus coeruleus are probably derived from the BST. The oxytocin innervation of the brain seems to be derived exclusively from the PVN⁸.

Immunoelectronmicroscopy has revealed that AVP and OXT fibers terminate synaptically^{2,38}. In addition, AVP and OXT were released in vitro from extrahypothalamic regions upon a depolarizing stimulus³. These findings, in combination with observations demonstrating central effects of AVP and OXT on processes such as temperature regulation⁶, fluid and electrolyte homeostasis and cognitive functions (for a review see ref. 13) suggested that these neuropeptides may be involved as neurotransmitters in a number of central functions.

Because many of the processes mentioned above show changes during aging (e.g. ref. 15), the integrity of neuroendocrine and central AVP systems in senescence has been questioned. This idea was reinforced since administration of exogenous AVP was reported to restore some of the behavioral deficits observed in aged animals and man (e.g. refs. 7, 24), leading to a number of clinical trials with AVP in memory disorders (for a review see ref. 21).

With respect to the integrity of the classical AVP-containing hypothalamo-neurohypophyseal system

(HNS), a decline of neurosecretory function with aging has been postulated^{26,34}. Recently, however, an increased HNS activity was found in the aged rat^{16,18}, probably due to a decrease of AVP binding sites in the renal collecting ducts³³. Concerning the integrity of central AVP pathways, Dorsa and Bottemiller¹⁴ reported decreased AVP concentrations as measured by radioimmunoassay (RIA) in a number of intra- and extrahypothalamic areas in aged rats, e.g. in the septum, the vascular organ of the lamina terminalis and the locus coeruleus. Also in the human brain, the neurosecretory AVP cells appear to be activated in senescence^{17,32}. However, no significant changes with aging have been found in extrahypothalamic AVP concentrations in the human brain^{20,27}.

Radioimmunoassay, however, does not necessarily give information with respect to changes in fiber density. In order to investigate whether morphological correlates could be found for the hypothesized deterioration of the central AVP system in senescence, the AVP and OXT innervation of the brain was investigated in the present study by means of immunocytochemistry in rats of two different age groups.

MATERIALS AND METHODS

Twelve male Brown-Norway rats (TNO/IVEG, Rijswijk, The Netherlands) in two different age groups (5 months ($n = 6$) and 33–35 months ($n = 6$) of age) were used. The animals were anesthetized intraperitoneally with sodium pentobarbital (Nembutal; 0.1 ml/100 g b.wt.) and perfused intracardially with 0.9% saline, followed by 5% glutaraldehyde (Merck) in 0.1 M cacodylate buffer, pH 7.5. The brains were dissected, immersed in the same fixative for an additional 6 h at 4 °C and stored in 0.05 M Tris (Sigma; pH 7.5). Subsequently, vibratome (Lancer) sections of 50 μ m were cut transversally.

Immunocytochemical staining consisted of simultaneous incubation of free-floating sections from young and old animals in the following solutions: (a) washing in 0.05 M Tris, pH 7.6, containing 0.9% NaCl and 0.5% Triton X-100 (Tris-Triton) (40 min); (b) rabbit AVP-antiserum (W-1) 1:800 or OXT-antiserum (O-1-V) 1:1000 in Tris-Triton (overnight at 4 °C); (c) washing in Tris-Triton (40 min); (d) goat anti-rabbit IgG serum (Betsie) 1:50 in Tris-Triton (90

min); (e) washing in Tris-Triton (40 min); (f) peroxidase anti-peroxidase (PAP) 1:1000 in Tris-Triton (90 min); (g) washing in Tris-Triton (20 min); (h) washing in 0.05 M Tris-HCl, pH 7.6 (20 min); (i) 0.5 mg/ml 3,3'-diaminobenzidine (DAB; Sigma) in 0.05 M Tris-HCl, pH 7.6, 0.01% H₂O₂ (20 min).

After staining the sections were washed in aquadest, mounted on glass slides and dried overnight, dehydrated and coverslipped with Entellan (Merck).

AVP and OXT fiber density was investigated in a number of brain areas, the nomenclature of which was adopted from Paxinos and Watson²⁵. The same atlas was used to select the area of AVP and OXT innervation within the various anatomical structures (indicated in parentheses as the anteroposterior distance from bregma): the vertical limb of the diagonal band (+0.7 mm), the lateral septum (−0.3 mm), the basal nucleus of Meynert (−2.3 mm), the paraventricular thalamic nucleus (−2.8 mm), the dorsomedial hypothalamic nucleus (−3.3 mm), the medial amygdaloid nucleus (−3.3 mm), the lateral habenular nucleus (−3.8 mm), the substantia nigra, pars compacta (−5.8 mm), the ventral part of the hippocampus (−5.8 mm), the central gray (−5.8 mm), the locus coeruleus (−9.3 mm), the nucleus of the solitary tract (−11.8 mm) and the ambiguous nucleus (−11.8 mm). From the center of each area, one section per animal was selected and fiber density was estimated by 3 investigators. Coded sections were ranked from low to high fiber density for each area separately. Subsequently, the sample distribution of ranks for young and old animals was tested for each area by means of the Mann-Whitney test (M-W; two-tailed and corrected for ties; 0.05 level of significance).

RESULTS

In general, the individual variation of extrahypothalamic AVP fiber density was larger in the aged animals than in the young group.

A marked decrease in AVP fiber density in old rats was observed in the vertical limb of the diagonal band, the basal nucleus of Meynert (Fig. 1), the lateral habenular nucleus (Fig. 2), the medial amygdaloid nucleus (Fig. 1), the substantia nigra, the ventral hippocampus, the central gray, the locus coeruleus (Fig. 3) and the ambiguous nucleus (see Table I). In these

Fig. 1. Transverse sections of the medial amygdaloid nucleus (MA) and basal nucleus of Meynert (B) of a young (A) and old (B) rat. Note the low AVP fiber density in both areas in the old animal. Arrows point towards weakly staining AVP immunoreactive cells, that were found in both areas in young animals only. OT, optic tract; ST, stria terminalis; Bar = 100 μ m.

Fig. 2. Transverse sections of the lateral habenular (LH) and paraventricular thalamic (PV) nucleus of a young (A) and old (B) rat. Note the strong decrease of AVP fiber density in the lateral habenular nucleus of the old rat, whereas the AVP fiber density in the paraventricular nucleus is not different. v, third ventricle; Bar = 100 μ m.

areas, not only the density of AVP fibers, but also their staining intensity was reduced. AVP fiber density was moderately reduced in the lateral septum (Fig. 4) and the dorsomedial hypothalamic nucleus of the aged animals. No age differences were observed in AVP fiber density in the paraventricular thalamic nucleus (Fig. 2) and in the nucleus of the solitary tract (see Table I).

In one old animal, practically no AVP fibers at all were observed in the vertical limb of the diagonal band, the lateral septum (Fig. 4), the basal nucleus of Meynert, the medial amygdaloid nucleus, the lateral habenular nucleus, the substantia nigra, the hippocampus, the central gray and the locus coeruleus. In contrast, the AVP fiber density in the paraventricular thalamic nucleus, the dorsomedial hypothalamic nucleus and the nucleus of the solitary tract in this animal did not differ from other young and aged animals.

Extrahypothalamic AVP immunoreactive cell

bodies were observed in the medial amygdaloid nucleus, and, occasionally, in the basal nucleus of Meynert only of young animals (Fig. 1).

No difference in OXT fiber density between young and old animals was observed in the locus coeruleus (Fig. 3), the nucleus of the solitary tract and the ambiguous nucleus (see Table I).

DISCUSSION

In the present study, differential changes with aging in AVP, but not in OXT fiber density were found in a number of extrahypothalamic areas. The fact that in those areas with the highest density of AVP fibers, such as the lateral septum and the lateral habenular nucleus, practically no fibers could be distinguished with anti-OXT staining demonstrates the absence of cross-reactivity of the anti-OXT serum in the immunocytochemical procedure used. In addition, cross-reactivity of the anti-AVP serum for OXT

Fig. 3. Transverse sections of the locus coeruleus (lc) of a young (A and C) and old (B and D) rat. The upper photographs show the AVP fibers, the lower photographs show the OXT fibers. Note the decrease in AVP fiber density in the old animal as compared with the young one and the different localization of OXT fibers as compared with AVP fibers. OXT fiber density does not differ between young and old animals. v, fourth ventricle; Bar = 100 μ m.

Fig. 4. Transverse sections of the lateral septum (LS) of a young (A) and old (B) rat. The right photograph (C) shows the lateral septum of an aged rat that did not show any AVP immunoreactive fibers in its lateral septum. V, lateral ventricle; Bar = 100 μ m.

TABLE I

The difference in AVP and OXT fiber density between young and old animals in a number of brain areas and the possible source of these fibers

Amb, ambiguous nucleus; B, basal nucleus of Meynert; BST, bed nucleus of the stria terminalis; CG, central gray; DM, dorsomedial hypothalamic nucleus; Hi, hippocampus; LC, locus coeruleus; LH, lateral habenular nucleus; LS, lateral septum; MA, medial amygdaloid nucleus; PV, paraventricular thalamic nucleus; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SNC, substantia nigra, pars compacta; Sol, nucleus of the solitary tract; VDB, vertical limb of Broca's diagonal band.

Probable source	Site of termination	Observed change	P-value	Testosterone dependency**
<i>AVP fibers</i>				
SCN	DM	Moderate decrease	0.0828	—
	PV	No change	1.0000	—
PVN	Sol	No change	0.5233	—
	Amb	Decrease	0.0433*	—
BST	VDB	Decrease	0.0051*	+
	LS	Moderate decrease	0.0828	+
	LH	Decrease	0.0147*	+
	CG	Decrease	0.0013*	+
	LC	Decrease	0.0057*	+
Unknown	B	Decrease	0.0089*	+
	Hi	Decrease	0.0067*	+
	MA	Decrease	0.0062*	+
	SNC	Decrease	0.0013*	+
<i>OXT fibers</i>				
PVN	Sol	No change	0.3613	—
	Amb	No change	0.8918	—
	LC	No change	0.3637	—

* Statistically significant (Mann–Whitney; two-tailed and corrected for ties; $P < 0.05$) difference in fiber density between the young and old group resulting from a ranking of fiber density.

** cf. De Vries et al.¹².

was also negligible, since in the locus coeruleus, where both AVP and OXT fibers are present, AVP and OXT fibers were differentially localized (see Fig. 3). However, ultimate proof for the specificity of the immunocytochemical staining in those areas where both AVP and OXT fibers are present cannot be given.

The observed decrease of AVP fiber density in the locus coeruleus is in agreement with the decline in AVP concentrations as measured by radioimmunoassay in this area by Dorsa and Bottemiller¹⁴. The same holds true for the decrease in AVP innervation of the lateral septum, although it was not statistically significant in our observations. However, the organum vasculosum of the lamina terminalis, another area where these authors observed alterations in AVP content, could not be reliably judged in our preparations.

There is a remarkable similarity in decreasing AVP fiber density with aging as demonstrated in the present study, and decreased AVP innervation following castration either during development or in adulthood^{9,10}. Castration results in loss of those AVP fibers that are most probably derived from the BST. In addition, AVP fibers disappear following castration in some areas the source of which is unknown, e.g. the medial amygdaloid nucleus, ventral hippocampus and n. basalis¹². The parallel with the condition after castration was extremely obvious in one old rat, that showed a complete disappearance of AVP fibers, e.g. in the lateral septum, the lateral habenular nucleus and the locus coeruleus, without any apparent change in AVP fiber density in the paraventricular thalamic nucleus and the nucleus of the solitary tract. In view of this parallel and since testosterone levels decrease during aging in male rats of various strains^{5,22}, the observed decrease in AVP fiber density in senescence might at least be explained in part by age-related decline in testosterone levels. Interestingly enough, decreased levels of testosterone might also be of importance for the age-related changes that have been observed earlier in the activity of the HNS¹⁶, since castration of adult male rats has been shown to induce an increase in neurosecretory activity^{29,30}.

Two changes with aging that were observed in AVP fiber density are not similar to those found following castration (cf. ref. 11), i.e. a decrease in the

ambiguous nucleus and a non-significant decrease in the dorsomedial hypothalamic nucleus innervation. The AVP innervation in these areas is derived from the PVN⁸ and the SCN¹⁹, respectively.

The slight change in the dorsomedial hypothalamic nucleus points to an alteration in the SCN in the old rat. In the human SCN a marked decrease in volume and cell number was recently found in subjects over 80 years of age³². The decrease in amplitude of circadian rhythms in the aged rat³⁶ suggests that degenerative changes with aging might also take place in the rat SCN. The decrease in AVP fiber density in the ambiguous nucleus suggests that changes with aging occur in the PVN. In this nucleus we have observed an activation of AVP neurons both in aged Wistar rats¹⁶ and in senescent human brain^{17,32}. It should be noted, however, that these latter investigations dealt mainly with the neurosecretory part of the PVN.

AVP immunoreactive cell bodies outside the hypothalamus were frequently observed in the medial amygdaloid nucleus and occasionally in the basal nucleus of Meynert in young animals, but not in the aged group. Again, the immunocytochemical stainability of these AVP immunoreactive cells has also been shown to be dependent on the level of circulating androgens¹². No apparent age-differences in stainability were observed in the supraoptic, paraventricular and suprachiasmatic nucleus, although the AVP cells in the SON and PVN are activated in the aged rat¹⁶.

It can be concluded from the present study, that no generalized changes appear to occur in the central AVP systems with aging. The meaning of the observed differential changes in immunocytochemical staining of AVP fibers between young and old animals is not easy to explain, since decreased AVP fiber density as demonstrated by immunocytochemistry may be due to a decrease either in the number of AVP fibers, or to a condition in which the fibers are still present but contain less AVP. On the other hand, the very similar changes observed following castration can most probably be interpreted as a decrease in AVP production in the affected areas¹². It may be of interest that in the present study a decrease in vasopressin innervation was found in a number of brain areas that show remarkable age-related changes, e.g. the hippocampus and the basal nucleus of Meynert^{1,23}. Since in adulthood long-term testosterone

treatment has been shown to reverse the decrease in vasopressin innervation following castration, a study of the effects of testosterone treatment in senescence seems to be an interesting line for future research.

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