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Extrahypothalamic vasopressin and oxytocin in the human brain; presence of vasopressin cells in the bed nucleus of the stria terminalis

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In the present study, the distribution of extrahypothalamic vasopressin (VP) and oxytocin (OXT) in the human brain was investigated by means of immunocytochemistry. In the septum verum, few VP fibers were found in the nucleus septalis lateralis and medialis (NSL and NSM), and in the bed nucleus of the anterior commissure. Very few VP and OXT fibers were present in the amygdala and in the hippocampus, mainly around the rostral tip of the lateral ventricle on the level of the pes hippocampi. The locus coeruleus (LC) contained dense networks of VP fibers and, although to a lesser extent, OXT fibers over its entire rostrocaudal extension. VP-immunoreactive neurons were present in the bed nucleus of the stria terminalis in a number of subjects, while no OXT cells were found in this structure. Thus, the VP innervation of limbic structures in the human brain, in particular of the NSL, was found to be clearly less pronounced than in the rat brain. The VP innervation of the LC, by contrast, was denser in the human brain than in the rat brain. No sex differences were found in the VP innervation of the human brain. These findings stress the need for caution in extrapolation of data concerning peptidergic innervation of the rat brain towards the human brain.

Vasopressin (VP)-synthesizing neurons have been found in the paraventricular nucleus (PVN), supraoptic nucleus (SON) and in the suprachiasmatic nucleus (SCN) of the rat¹⁹. Only in the PVN and SON, neurons are found which produce oxytocin (OXT). A similar localization was found in the human brain^{7,9,20}. Additional VP neurons were found in the bed nucleus of the stria terminalis (BST), the dorsomedial hypothalamic nucleus (DMH), locus coeruleus (LC) and amygdala of rats that were pretreated with colchicine^{4,21}. Such cell bodies have not yet been described in the human brain.

VP and OXT neurons of the SON and PVN project as neurosecretory neurons to the neurohypophysis, where VP and OXT are released into the circulation. Immunocytochemical studies in rats have shown that VP- and OXT-containing fibers are also present outside the hypothalamo-neurohypophyseal system, where VP and OXT are probably released as neurotransmitters from synaptic terminals (for a review see ref. 3). VP and OXT fibers were also demonstrated in the human brainstem and spinal cord¹⁷, while their

presence was observed already by 17 weeks of gestation in the medulla oblongata and septum¹⁸. Also by means of radioimmunoassay (RIA), VP has been detected in a number of areas in the human brain^{13,16}.

These extensive VP and OXT fiber projections are considered to be the anatomical substrate for the effects of these neuropeptides on the central nervous system. VP has been proposed to be involved for example in temperature regulation⁵ and in learning and memory (e.g. ref. 2), while OXT has been reported to impair memory processes⁸ and to elicit maternal behaviour¹⁴.

Recently, we found changes in the VP-containing neurons in aging and Alzheimer's disease that differed with the brain area studied: VP cells in the SON and PVN showed signs of increased activity in senescence and in Alzheimer's disease^{9,12}, while total cell numbers remained stable in these areas¹¹. However, in the human SCN a loss of VP cells was found in senescence and even more so in Alzheimer's disease²⁰. A differential pattern of age-related changes was also observed in the VP innervation of the rat brain. A

TABLE I

Brain material

<i>Patient</i>	<i>No.</i>	<i>Sex</i>	<i>Age (years)</i>	<i>Postmortem delay (h)</i>	<i>Brain weight (g)</i>	<i>Clinical diagnosis</i>
(1)	82173	m	18	25	1350	multiple traumata; rupture of aorta
(2)	82115	m	36	14	1670	acute myocardial infarction
(3)	82097	f	45	10	1350	metastatic mamma carcinoma
(4)	82077	m	64	6	1450	metastatic bronchus carcinoma; polycystic kidneys
(5)	84257	m	64	*	1430	renal failure; dehydration
(6)	82101	f	70	25	1240	gastric carcinoma; pulmonary embolism
(7)	82242	m	72	*	1450	*
(8)	82313	f	72	10	1140	mesenteric vascular occlusion
(9)	82278	m	74	12	1400	bronchogenic carcinoma
(10)	82056	m	74	61	1340	cervical trauma
(11)	82136	f	74	16	1340	metastatic endometrial carcinoma
(12)	82114	m	79	21	1300	hypovolemic shock
(13)	82121	f	90	15	1030	acute pancreatitis

* Information not available.

marked reduction in VP fiber density was found in those areas where the VP innervation had previously been shown to be dependent on the levels of circulating androgens¹⁰.

At present, little information is available about the morphological distribution of VP and OXT fibers in the human brain, since most of the studies so far were performed by RIA (e.g. refs. 13 and 16). In order to compare the distribution of VP fibers with that in the rat brain and to provide the basic information needed for a study on changes in aging and Alzheimer's disease, the VP and OXT fiber distribution in the human brain was investigated by means of immunocytochemistry. In addition, the possible presence of VP-immunoreactive neurons was investigated in those extrahypothalamic areas which have recently been found to contain VP cells in the colchicine-pre-treated rat.

For the present study, brains of 13 subjects without neurological diseases were obtained at autopsy (see Table I). Within 2 h after autopsy, the septal area, amygdala, hippocampus and LC were dissected from unfixed brains. The tissue blocks were immersed in 1% paraformaldehyde (Merck) and 2.5% glutaraldehyde (Merck) in 0.1 M sodium cacodylate buffer (Fluka AG) at pH 7.4 and kept at 4 °C under constant agitation for 7 days. Subsequently, the tissue blocks were frozen and stored at -80 °C. Cryostat sections (40 µm) were cut serially at -8 °C, mounted on uncoated glass slides and stored at -20 °C. For orientation, every 20th section was mounted on albu-

minized slides and stained with thionin (0.1% thionin in acetate buffer, pH 4; 20 min). In one case (subject no. 5; see Table I) the fixed material was not frozen but processed by 40 µm sectioning on a vibratome and stored at 4 °C.

Before immunocytochemical staining with VP- and OXT-antiserum, alternating frozen sections (one pair, each 200–600 µm) were separated from the glass slides with a paintbrush in a cold (4 °C) Tris-NaCl buffer (0.05 M Tris (Sigma)–0.5 M NaCl (Baker), pH 8.6). Consequently, both cryostat and vibratome sections could be incubated floating free, which made the procedure more sensitive. The incubations were performed in plastic vials at room temperature under constant agitation, unless stated otherwise. The staining procedure was as described in ref. 10, except for the first steps to remove pseudoperoxidase activity: rinsing in Tris-NaCl (2 × 30 min), followed by rinsing in 50% methanol (Baker; 15 min), 100% methanol (30 min) and 50% methanol (15 min). As first antisera were used: (a) VP-antiserum (no. 126, 24-12-72 or W1; 1:800); (b) OXT-antiserum (0-1-V, 4-4-75; 1:1000). In order to exclude staining by cross-reactivity, VP- and OXT-adsorbed antisera were used in those areas where both VP- and OXT-fiber staining was observed (for details on the adsorptions see ref. 15): (c) OXT-adsorbed VP-antiserum (126-OXT; 1:200); (d) VP-adsorbed OXT-antiserum (0-1-V-VP; 1:400); or as controls: (e) pre-immune serum (Hermanus, 26-6-80, 1:800; or 0-1-CTRL, 1:1000), both leading to complete absence of staining.

Fig. 1. Transverse cryostat sections, stained for VP, of (a) the NSM and (b) the NSL of a 90-year-old woman (subject no. 13; see Table I). Note the paucity of fibers in the NSL and the slightly higher fiber density in the NSM. LV, lateral ventricle; bar = 100 μ m. c: the LC of a 74-year-old man (subject no. 9; see Table I). d: detail from c. Note the dense VP fiber networks in the LC between the neuromelanin-containing cell bodies. Arrow points towards structure, suggestive of perineuronal termination. V, fourth ventricle; bars represent 100 μ m (a) and 50 μ m (b), respectively.

VP and OXT fiber innervation of septal nuclei was examined in subjects nos. 1–4, 6, 10–13 (see Table I). In the nucleus septalis medialis (NSM), BST and nucleus of Broca's diagonal band (NBDB) both VP and OXT fibers were found. In the NSM, only staining with VP-antiserum was found when purified antisera were used, while in the other two structures no cross-reactivity was observed.

The highest incidence of VP fibers in the limbic system was found in the BST, NSM, NBDB and bed nucleus of the anterior commissure. In the BST both beaded and unbeaded fibers were observed. The NSM showed a higher fiber density than the nucleus septalis lateralis (NSL) (Fig. 1a, b). No sex difference in VP innervation was observed in any of the septal regions investigated. Some VP fibers were also observed in structures adjacent to the septum, i.e. the nucleus caudatus and the capsula interna. In ad-

dition, occasional OXT fibers were found in the nucleus accumbens septi.

In the hippocampus and amygdala (subjects nos. 1, 3, 6–10; see Table I), very few VP and OXT fibers were found solely around the rostral tip of the lateral ventricle on the level of the pes hippocampi. No immunoreactive cell bodies were found in these structures.

The LC (subjects nos. 7–9; see Table I) was found to be very densely innervated by VP fibers over its entire rostrocaudal extension (Fig. 1c, d). In addition, many OXT fibers were observed in this area. Structures suggestive of perineuronal termination were frequently observed in the LC, stained for VP.

In the BST, few VP-immunoreactive cells were found in subjects nos. 3 and 4 (see Table I). Rostrally, these cells were smaller than the magnocellular neurons of the PVN and SON, and more often bipo-

Fig. 2. Transverse vibratome section stained for VP through the PVN and the BST of a 64-year-old man (subject no. 5; see Table I). b: detail from a. Note the presence of immunoreactive neurons in the BST. Arrows point towards bipolar VP-immunoreactive neurons. LV, lateral ventricle; III, third ventricle; bars represent 200 μ m (a) and 100 μ m (b), respectively.

lar in shape. On a more caudal level, many VP-immunoreactive cells were found in subject no. 5, some of which were magnocellular (Fig. 2).

The dorsomedial hypothalamic nucleus was present only in subject no. 5, and did not contain VP-immunoreactive neurons.

In the present study, a differential visualization of VP and OXT fibers in the human septum, amygdala, hippocampus and LC was obtained by the use of purified VP and OXT antisera.

In the rat brain, fixed by perfusion, the VP innervation of the NSL is very dense and higher in males than in females⁶. The present study revealed that the VP innervation of the human septum is much less dense than that of the rat septum, while, in contrast to the rat, no sex difference was observed. It is unlikely that the low VP-fiber density in the human septum is caused by inadequate fixation, since proces-

sing of the LC from the same material revealed extensive networks of VP fibers, the density of which even exceeded the VP innervation of the rat LC. In addition, the low fiber density in the human septum is in accordance with the small quantities of VP in the human septum as measured by RIA¹⁶. Species differences in septal VP innervation may be of functional importance, for example with respect to cross-species differences in response to intraseptal administration of VP. For instance, VP has been reported to have an antipyretic effect in sheep⁵ but not in the rabbit¹.

In the rat, extrahypothalamic VP cells in the BST could be visualized best following colchicine pretreatment²¹, while, in addition, immunoreactivity of these cells has been shown to be dependent on the levels of circulating androgens⁶. In the present study, VP-immunoreactive neurons were observed in the

BST of 3 subjects. In this respect it is of interest that the only woman from our series in which VP cells were present in the BST (no. 3; see Table I) had been treated with anti-estrogens. Two male subjects in which VP cells could be visualized in the BST (nos. 4 and 5; see Table I) both had suffered from renal pathology. The possible functional significance of these observations is unknown at present.

Neurons in the LC contained neuromelanin, which was impossible to distinguish in our sections from diaminobenzidine deposits. Therefore, the possible presence of VP cells in the human LC, as described earlier in the rat⁴, can neither be confirmed nor excluded from the present study. The use of a different chromogen for the peroxidase reaction might be helpful in this respect.

An effect of age or sex on the density of immunocytochemically stained VP and OXT fibers was not found in the present study. Since such a difference may have been masked by the small number of specimens, it will be the subject of further investigation, in which material of patients with Alzheimer's disease will also be included.

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