Intra- and Extrahypothalamic Vasopressin and Oxytocin Pathways in the Rat

Pathways to the Limbic System, Medulla oblongata and Spinal Cord*

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Summary. Vasopressin and oxytocin pathways were specifically localized in glutaraldehyde-paraformaldehyde fixed rat brains, with the use of the unlabelled antibody enzyme method and purification of the first antiserum. Vasopressin and oxytocin containing pathways were traced from the paraventricular nucleus towards the dorsal and ventral hippocampus, the nuclei of the amygdala, substantia nigra and substantia grisea, nucleus tractus solitarius, nucleus ambiguus and to the substantia gelatinosa of the spinal cord. In addition, a vasopressin containing pathway between the suprachiasmatic nucleus and the lateral habenular nucleus was demonstrated. The possible nature (axons or dendrites) and role of these extrahypothalamic fibres is discussed in relation to water balance, milk ejection and avoidance behaviour.

Key words: Vasopressin – Oxytocin – Immunohistochemistry – Water balance – Milk ejection.

Neurohypophysial hormones are synthesized in the supraoptic (SON), paraventricular (PVN) and, probably, the suprachiasmatic nucleus (SCN) (Swaab and Pool, 1975; Vandesande et al., 1975). The hormones vasopressin and oxytocin originate from the first two (magnocellular) nuclei and are transported via the hypothalamo-neurohypophysial tract to the neurohypophysis. However, since E. Scharrer (1951) demonstrated the existence of extrahypothalamic pathways, on the basis of Gomori staining in the garter snake, evidence has accumulated for the presence of neurosecretory pathways to various brain regions also in mammals. Such pathways, which are presently thought to be the anatomical basis for the behavioral effects of vasopressin (De Wied and Gispen, 1977; Buijs et al., 1978),

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have been described by the elaborate Gomori studies of Barry (1961), by Sterba (1967) using pseudo-isocyanine staining, and by Brownfield and Kozlowski (1976), Weindl et al. (1976), Swanson (1977) and Sofroniew and Weindl (1978), who all demonstrated neurophysins immunocytochemically. All these techniques, however, provided only indirect evidence for the neurosecretory nature of the material present in these pathways.

The vasopressin and oxytocin content of a number of extrahypothalamic pathways was substantiated by means of immunocytochemical methods (Buijs et al., 1978). In the present report the presence of hypothalamo-limbic pathways for vasopressin as well as oxytocin is extended; new connections are described between the SCN and the limbic system while, in addition, vasopressin and oxytocin fibres are demonstrated in a large number of other brain areas ranging from the entorhinal cortex to the spinal cord.

Materials and Methods

Six male Wistar rats and three male rats of the Brattleboro strain, homozygous for diabetes insipidus, (Ho-DI), were used. For details about animals, fixation and staining of the tissue, specificity controls, and production and purification of the antisera, the reader is referred to other papers (Buijs et al., 1978; Swaab and Pool, 1975). In principle, the fibres were stained by means of purified anti-vasopressin or -oxytocin plasma according to the unlabelled antibody enzyme method (Sternberger, 1974). Initially, however, a mixture of anti-oxytocin ( = 02C) and anti-vasopressin ( = 125 ) (1:200 and 1:400 final dilution, respectively) was used to trace neurohypophysial-hormone-containing fibres in sagittal and transverse brain sections. The neurosecretory content of the fibres was subsequently indicated in alternating sections with the purified antisera.

Results

The Suprachiasmatic Nucleus

From this parvocellular nucleus, fibres could be visualized with purified anti-vasopressin plasma, fanning out into various directions. As has already been reported (Buijs et al., 1978), fibres from the SCN reach the organum vasculosum laminae terminalis (OVLT), the periventricular nucleus (pvs) and the lateral septum (sl), while fibres also project to the SON (Figs. 1–3). The dorsally directed fibres could now be followed in the periventricular nucleus running just under the ependyma of the lateral ventricle (Fig.1). These fibres seem to terminate in the lateral habenular nucleus (l.h.), as indicated by punctate perineural varicosities (Figs. 4, 6a). No oxytocin containing fibres were found in the lateral habenular nucleus (Fig. 6b). The exclusive presence of vasopressin fibres in this area is also supported by the fact that no neurohypophysial hormone containing fibres at all could be demonstrated in this region in the Brattleboro rats. Some fibres from the periventricular or lateral habenular nucleus seem either to continue into the superior colliculus (SC) or to descend via the fasciculus retroflexus (FR) towards the interpeduncular nucleus (ip) (Figs. 1, 5). In addition, vasopressin containing fibres were sometimes demonstrated in the subcommissural organ and a single fibre was found in the stalk of the pineal.
The Supraoptic Nucleus

A possible contribution of SON fibres to extrahypothalamic pathways arising from the PVN can neither be excluded nor established, because of the intermingling of the fibres of these two nuclei. Consequently, if the PVN is mentioned as the source of extrahypothalamic fibres, the possibility of the SON as a (partial) contributor is still left open.

The Paraventricular Nucleus

In the PVN frequently bipolar and even multipolar neurosecretory cells were observed, having fibres that run into different directions. Incubating sagittal sections, a large number of vasopressin and oxytocin containing extrahypothalamic pathways could be revealed. In the description of these pathways the numbering corresponds with the numbers in Figs. 1 and 3.

(I) From the PVN, vasopressin and oxytocin containing fibres follow the fornix dorso-rostrally into the dorsal hippocampus and subiculum (S) via the ventral commissure of the fornix (CFV) just passing the subfornical organ in which scarce vasopressin and oxytocin fibres are visible. From the dorsal hippocampus the fibres continue into the ventral hippocampus via the fimbriae hippocampi (FH) (Figs. 4, 5, 7). In addition, a few vasopressin and oxytocin fibres can be followed via the tapetum (T) into the entorhinal cortex (CE).

(II) Other fibres course rostrally, pass the anterior commissure ventrally, and reach the medial and the lateral septum. Especially in the lateral and the dorsal septum a large number of vasopressin containing fibres are present, ending with pericellular punctate structures (Fig. 2).

(III) Another tract courses into a rostro-ventral direction parallel to the nervus opticus, and can be followed to the caudal part of the tractus diagonalis (TD). Its exact site of termination could not be ascertained, however.

(IV) Vasopressin and oxytocin fibres run from the PVN caudally to the medulla oblongata via the fibrae periventriculares hypothalami, the fasciculus longitudinalis dorsalis (FLD) and substantia grisea centralis (SGC) (the bundle of Schütz) (Figs. 1, 5).

(V) The other directly caudally projecting tract of the PVN runs via the fasciculus retroflexus, interpeduncular nucleus, substantia nigra (SNR) and the lateral lemniscus into the medulla oblongata.

(VI) Vasopressin and oxytocin containing fibres run via the infundibular recess through the arcuate nucleus, and subsequently in a caudo-lateral direction (Fig. 8) into the reticular zone of the substantia nigra (Figs. 5, 9); some of these fibres reach the medulla oblongata via the formatio reticularis and the lateral lemniscus.

Especially in transverse sections the pathways to the stria medullaris (VII) and the stria terminalis (VIII), which have been reported before (Buijs et al., 1978) can be readily visualized (Figs. 1, 3). Vasopressin and oxytocin fibres were found in the present study to travel via the stria terminalis to the medial, central, lateral and basal nuclei of the amygdala (Fig. 4) where they terminate with punctate perineural structures.
Figs. 1–5. Diagrams illustrating pathways and possible sites of termination in sagittal and transverse planes reproduced from stereotaxic atlas of König and Klippel (1963). Vasopressin and oxytocin fibres shown as thin lines; sites of termination indicated by semicircles. Magnocellular nuclei PVN and SON indicated by large dots, parvocellular nucleus SCN indicated by medium dots. Transverse sections arranged from rostral to caudal. CA anterior commissure; CAI Capsula interna; CE entorhinal cortex; CFV ventral commissure of fornix; CO optic chiasm; F fornix; FH fimbria hippocampi; FLD fasciculus longitudinalis dorsalis; FOR formatio reticularis; FR fasciculus retroflexus; Hi hippocampus; LM lemniscus medialis; LV lateral ventricle; 3V third ventricle; OVLT organum vasculosum laminae terminalis; PVN paraventricular nucleus; S subiculum; SC superior colliculus; SCN suprachiasmatic nucleus; SGC substantia grisea centralis; SM stria medullaris; SNR substantia nigra; SON supraoptic nucleus; ST stria terminalis; T tapetum; TCC trunclus corpus callosi; TD tractus diagonalis; abl lateral basal nucleus of amygdala; am medial nucleus of amygdala; cc central canal; cp nucleus caudatus; cwr Edinger-Westphal nucleus; ip interpeduncular nucleus; lh lateral habenular nucleus; mb medial habenular nucleus; pvr and pvs periventricular nucleus; sl lateral septum; sm medial septum; tam anterior thalamic nucleus

Vasopressin and oxytocin fibres are found scattered throughout the formatio reticularis, and descend from the fasciculus longitudinalis dorsalis to ventral via the Edinger-Westphal nuclei and the dorsal and medial raphe nuclei. In addition, these neurosecretory fibres were demonstrated in the nucleus parafascicularis and superior colliculus. As a rule, far more vasopressin than oxytocin fibres are seen in the diencephalic and mesencephalic subcortical brain regions; especially in the nuclei of the amygdala and in the ventral hippocampus most fibres are vasopressinergic.

Vasopressin and oxytocin fibres can also be visualized in various regions of the medulla oblongata. Many fibres course from ventral to dorsal, or vice versa, along
Fig. 6 A and B. Transverse sections of Wistar rat brain in region of lateral habenular nucleus. A Fibres as revealed by purified vasopressin antiserum 1:200. Note pericellular punctate structures in periventricular nucleus and lateral habenular nucleus (arrows), and some fibres descending with fasciculus retroflexus. × 350. B One of following sections incubated with purified oxytocin antiserum (1:200). Note complete absence of staining. × 125
Figs. 7 and 8. Transverse sections of Wistar rat brain. Fig. 7. Branching fibres (arrows) in apical dendritic tree of pyramidal layer in ventral hippocampus as revealed by purified vasopressin antiserum 1:200. \(\times 450\). Fig. 8. Results of incubation with nonpurified vasopressin antiserum (1:1000). Vasopressin fibres leaving arcuate nucleus coursing in lateral direction; neurohypophysial stalk already formed. \(\times 250\)
large blood vessels in the region of the formatio reticularis. A few vasopressin and oxytocin fibres are visible in the locus coeruleus, but a higher density of fibres was seen in the nucleus ambiguus and, even more so, in the nucleus tractus solitarius. From the latter nucleus the fibres course along the central canal into the spinal cord. In contrast with the more rostral brain regions, far more oxytocin than vasopressin fibres were found in the medulla oblongata as well as the spinal cord.
In the spinal cord these fibres are located mostly around the central canal and in the dorsal horn within the substantia gelatinosa (Fig. 11). Scarcely a fibre could be demonstrated in the ventral horn. In sagittal sections of the spinal cord these fibres appear to course rostro-caudally along the border of the grey and white matter and in the outermost laminae of the substantia gelatinosa and Lissauer tract.

Incubation without any antisera revealed only in some additional areas the same staining as with antisera. Such nonspecific staining was obtained in cells around the posterior part of the third ventricle, around the aquaeductus Sylvii, in the area postrema and at the border of the subfornical organ (Fig. 10).

The staining results in Ho-DI brain sections were generally the same as in normal Wistar brain sections with purified oxytocin antiserum, except that an occasional oxytocin containing fibre was seen in the area postrema of Ho-DI rats.

**Discussion**

Barry, in his extensive report of 1961, already described the localization of “Gomori-positive” fibres of magnocellular origin in several brain regions in various vertebrates. The distribution of vasopressin and oxytocin fibres in extrahypothalamic regions, as reported in the present paper, is in close correspondence with his
results. The advantage of immunohistochemical localization of these neuropeptides is the specific demonstration of vasopressin versus oxytocin, whereas Gomori-staining reveals peptide containing structures in general. This could be one of the reasons why reports dealing with either the Gomori technique or other merely selective staining procedures (Sterba, 1967) have attracted relatively little attention until recently. That the Gomori procedure also stains some structures that do not contain neurohypophyseal nonapeptides is demonstrated, for example, by the staining of cells of the subfornical organ, the area postrema and around the caudal part of the third and fourth ventricles (Vigh et al., 1963; Teichmann, 1967; Goldgeifer, 1976), all of these being structures in which no neurohypophyseal peptides were found with immunocytochemical methods. Furthermore, false negative results of Gomori staining can be deduced from the fact that only Joussen (1970) reported positive staining of the SCN in the rabbit with this technique, while no previous reports of such staining in the rat SCN are known to us. In addition, the connection between the SCN and the lateral habenular nucleus has never been reported before.

The distribution of the oxytocin and vasopressin containing fibres found with immunocytochemical methods appears to be in good agreement with data on their presence in certain brain regions as measured by means of radioimmunoassay (Dogterom, submitted). This supports the specificity of the immunocytochemical localization methods used.

Although little is known about the physiological function of the widespread oxytocin and vasopressin fibre system in the rat, a number of obvious possibilities exist. For instance the vasopressin fibres might be involved in the maintenance of water balance. The regions under consideration in this mechanism are also those where a large number of vasopressin fibres (all derived from the SCN) are found: the OVLT, the periventricular nucleus and the habenular nucleus. Lesioning of the anterior periventricular nucleus along the third and lateral ventricles induced inappropriate adaptation of vasopressin release during salt loading in the goat (Andersson et al., 1975) or during hydration in the rat (Buggy et al., 1977). In addition, it is reported that latero-caudal and dorsal isolation of the SCN pathways abolishes drinking rhythms (Nunez and Stephan, 1977). The SCN might thus be involved in drinking and regulation of the release of neurohypophysial hormones, e.g., via the vasopressin fibres that run from the SCN towards the SON and PVN. This is all the more plausible since vasopressin administered to the SON inhibits spiking of neurosecretory cells (Nicoll and Barker, 1971). Another important structure for the regulation of water balance in the brain is the choroid plexus. Vasopressin, or a vasopressin-like substance (Rudman and Chawla, 1976) present in the choroid plexus, may have a function in regulating the amount of CSF. Vasopressin may either be absorbed from the CSF (van Deurs et al., 1978) or the blood or directly transported to the plexus via a hypothalamo-choroidal tract (Brownfield and Kozlowski, 1977). In our material, however, vasopressin fibres were only seldom seen to enter the attachment place of the plexus (taenia choroidea thalami). The fibres running within the stria terminalis continue to the nuclei of the amygdala, which does not point to the existence of an extensive hypothalamo-choroidal tract.

The bi- and multipolarity of PVN cells suggests the possibility that the same cell may project to extrahypothalamic areas as well as to the neurohypophysis. Whether
the extrahypothalamic fibres are "dendrites" with a receptive function, as supposed by Dierickx for the frog (1962), or "axons" transporting a secretory product is not known. Especially for the fibres in the lateral septum and lateral habenular nucleus, which are derived from the SCN, the question arises whether these are axons or dendrites.

The wide-spread fibre system demonstrated is in line with the results on the afferent pathways controlling the release of neurohypophysial hormones as determined by electrical stimulation (Cross and Dyball, 1974). Such release occurs after stimulation of the spinal cord, tractus solitarius, bundle of Schütz, amygdala, septum, fornix, etc. Since we found vasopressin and oxytocin fibres in all these regions, such release after electrical stimulation need not necessarily be induced via indirect synaptic pathways but might be the result of direct stimulation of neurosecretory neurons.

The substantia gelatinosa in the spinal cord serves as a gate for peripheral information to higher centers of the brain (Wall, 1964; Melzack and Wall, 1965). The large number of oxytocin fibres in this part of the CNS opens the possibility that sensory information for the HNS, e.g., for the milk ejection reflex, might be picked up monosynaptically by dendrites in this part of the spinal cord.

Although it cannot be excluded that at least a part of the fibres in the lateral habenular nucleus, lateral septum and medial amygdala are dendritic in character, the punctate pericellular fibre structures in these areas suggest that neurohypophysial peptides are released within the limbic system. However, the nature of such synaptoid structures has still to be determined by conventional and immunohistochemical electron microscopy.

These neuropeptides could function as neurotransmitters influencing membrane properties (Barker, 1977) or as modulators of monoamine metabolism (Kovacs et al., 1977; Tanaka et al., 1977). This could be the basis for the demonstrated vasopressin effect on behavior (de Wied, 1965). This peptide, injected either systemically or into the CSF, caused a prolongation of extinction in an active avoidance test and an improvement of retention in passive avoidance behavior, while vasopressin antibodies injected intracerebroventricularly had the opposite effect (de Wied and Gispen, 1977; van Wimersma Greidanus et al., 1975).

The presence of vasopressin and oxytocin in the CSF independent of blood levels (Zaidi and Heller, 1974; Dogterom et al., 1977) argues for a direct central release of these peptides. In addition, lesions in the regions of punctate pericellular fibres cause behavioral deficits that are impervious to vasopressin treatment (van Wimersma Greidanus et al., 1976).

Although the CSF (de Wied and Gispen, 1977) is stressed as a transporting medium: for centrally active vasopressin, the present data, together with the lack of correlation between vasopressin-blood and-CSF levels and behavior (Dogterom, 1977; Dogterom et al., 1977), indicate that a direct transport via peptidergic neurosecretory fibres is the most likely possibility under physiological conditions.

What has to be taken into account also is that not vasopressin and oxytocin alone may influence brain function, but also the neurophysins (Pickering, 1976) or possibly their precursors (Gainer et al., 1977). To our knowledge their possible central effects have never been tested.

Even though the exact physiological function of centrally released vasopressin and oxytocin remains to be elucidated, the very existence of this extensive {
peptidergic fibre system supports the view that vasopressin and oxytocin are putative transmitters.

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