VASOPRESSIN AND OXYTOCIN, CHEMICAL MESSENGERS IN THE BRAIN

R.M. Buijs

Netherlands Institute for Brain Research
Ijdijk 28, 1095 KJ Amsterdam, the Netherlands

The neurohypophyseal hormones vasopressin and oxytocin are synthesized in the supraoptic (SON) and paraventricular nuclei (PVN), while the suprachiasmatic nucleus (SCN) contains vasopressin only (Swaab and Pool, 1975; Vandesande et al., 1975). These peptides are transported to the median eminence and the neurohypophysis where release into the blood occurs, where vasopressin acts primarily as an antidiuretic hormone on the kidney, while only in high doses it is effective on blood pressure. Oxytocin acts in the female on the mammary gland for the milk ejection and on the uterus especially during labor. In the male no clearcut peripheral function for oxytocin has been established up till now. Already in the thirties central effects for these hormones were suggested by Cushing (1932), in addition to the peripheral endocrine actions of vasopressin and oxytocin. He demonstrated pronounced central effects of an extract of the neurohypophysis following an injection into the cerebral ventricles of hypophysectomized patients. It was de Wied, however, who opened up the experimental field on the effects of pituitary hormones on the brain. It was demonstrated, e.g., that injection of vasopressin, either peripherally (de Wied, 1971) or centrally (Kovacs et al., 1979) resulted in the consolidation of acquired

behavior. In addition, vasopressin might be involved in the central regulation of water balance (Raichle and Grub, 1978), temperature (Cooper et al., 1979) or brain development (Boer et al., 1980).

By means of specific and sensitive immunocytochemical methods, a wide-spread vasopressin and oxytocin fiber system is demonstrated arising from the above mentioned hypothalamic nuclei. These peptidergic fibers reach brain regions ranging from the olfactory bulb to the spinal cord. In addition, from immunoelectronmicroscopical observations it appeared that these peptidergic fibers terminate on neurons and dendrites in the limbic system by means of synapses indistinguishable from the classical aminergic ones. These and other recent observations led to the conclusion that vasopressin and oxytocin most probably execute their central action via exohypothalamic fibers and might be regarded as peptidergic neurotransmitters.

Localization of vasopressin and oxytocin

Antisera against these peptides were raised and purified using agarose beads coupled with the heterologous peptide (Swaab and Pool, 1975). Rat brains were fixed by perfusion with 2.5% glutaraldehyde, 1% paraformaldehyde in 0.1 M cacodylate buffer pH 7.2 and sectioned on a vibratome for immunolight- or immunoelectron-microscopy, using the unlabeled antibody enzyme method (Sternberger, 1974). For further details of fixation, staining and specificity controls see Buijs et al. (1978); Buijs and Swaab (1979).

In the adult rat, vasopressin or oxytocin was demonstrated in cell bodies of the PVN and SON and in fibers running towards the neurohypophysis, as well as in cell bodies scattered throughout the hypothalamus. From the PVN or SON a great number of vasopressin or oxytocin fibers were found to run into many regions in the brain. It appeared that oxytocin fibers dominated in the caudal brain regions as the brain stem and spinal cord while mostly vasopressin fibers were demonstrated in the more rostral brain regions

of which especially the limbic system is densely innervated. In addition, also from the SCN a large number of only vasopressin containing fibers fan out into various directions (fig. 1).

Fig. 1 - Sagittal 100 μm thick vibratome section of the vasopressin containing parvocellular suprachiasmatic nucleus with fibers fanning out, (OC)= rostral part of the optic chiasm. Note the bipolar parvocellular neuron (arrow) and the magnocellular one (*) (Bar=25 μm).

However, as it appeared from lesion studies of the SCN (Hoorneman and Buijs, 1982) it is extremely difficult by immunocytochemical techniques alone to establish the source of the exohypothalamic fibers to a certain hypothalamic nucleus. From this lesion experiment followed by the immunocytochemical localization of vasopressin, it appeared that the SCN projects only to the organum vasculosum lamina terminalis, dorsomedial nucleus of the hypothalamus and periventricular nucleus (Hoorneman and Buijs, 1982). Consequently,

projections formerly ascribed to the SCN as the lateral septum, lateral habenular nucleus, diagonal band of Broca (Buijs et al., 1978; Buijs, 1978; Buijs, 1980; Sofroniew and Weindl, 1978a,b), amygdala, dorsal raphe nucleus and nucleus tractus solitarius

Fig. 2 - Transversal section with vasopressin containing fibers terminating in the region of the nucleus of the solitary tract (NTS) (AP=area postrema; NX=dorsal motor nucleus of the vagus; cc=central canal)

(Sofroniew and Weindl, 1978b) will be derived from the PVN or SON. Fig. 3 summarizes the main vasopressin pathways derived from the PVN or SON. In addition, it appeared that vasopressin fibers in the lateral septum terminate in a sex-dependent way, resulting in a much less innervation of this area in the female rat (de Vries et al., 1981).

Fig. 3 - illustrates pathways and possible sites of termination of vasopressin containing fibers in the rat brain projected in the sagittal plane. Paraventricular (PVN) and supraoptic nucleus (SON) are indicated by large triangles. 1, 2 or 3 interrupted lines are an indication for an increasing number of fibers in the pathway, while a λ indicates a possible site of termination.

A=amygdala; DBB=diagonal band of Broca; LC=locus coeruleus; LH=lateral habenular nucleus; MB=mammillary body; ME=median eminence; MFB=medial forebrain bundle; MS=medial septal nucleus; NA=nucleus ambiguus; NTS=nucleus of the solitary tract; NX=dorsal motor nucleus of the vagus; OB=olfactory bulb; P=pineal; PBD= dorsal parabrachial nucleus; PVG=periventricular grey; RD=dorsal raphe nucleus; RLM=lateral magnocellular reticular nucleus; SC=superior colliculus; SN=substantia nigra; VHi=ventral hippocampus

The synaptic localization of vasopressin and oxytocin

In order to verify whether vasopressin and oxytocin fibers really terminate in these brain regions, immunoelectronmicroscopy was performed on the most densely innervated structures, i.e. the lateral septum, lateral habenular nucleus and amygdala (Buijs and Swaab, 1979). In all three regions synaptic structures containing

vasopressin were found to terminate mostly on dendrites (fig. 4) but also on cell bodies. These synapses contained largely clear vesicles and infrequently dense core vesicles, the latter with a diameter of approximately 100 nm. These structures containing neurohypophyseal "hormones" were indistinguishable from classical neurotransmitter containing synapses, as visualized with a similar pre-embedding staining technique (e.g. Pickel et al., 1979).

Fig. 4 - Vasopressin containing terminal forming a synapse (arrow) with an unlabelled dendrite in the lateral septum. Note the DAB deposit around the clear vesicle-like structures. (Bar=0.25 μ m)

The observation that vasopressin and oxytocin are present in granules of 100 nm, does not fit in with the observation that the PVN and SON (where these fibers originate) contain granules of a larger size (140 nm)(van Leeuwen et al., 1978). Since in the neurohypophysis using this technique a granular size was found of 150 nm, it seems obvious that different cells project towards the neuro-

hypophysis and the brain or that a different release mechanism exists in central brain regions and neurohypophysis. Vasopressin immunoreactivity was frequently found at the surface of clear vesicle-like structures (fig. 4). This might fit in with the idea that the smooth endoplasmatic reticulum serves as an alternative vehicle for intra-axonal transport of non-granular neurosecretory material (Droz et al., 1973) that gives rise to clear vesicles in the axonal terminal (Alonso and Assenmacher, 1978).

Vasopressin and oxytocin; two peptide neurotransmitters?

The criteria for identification of a substance as a transmitter in the central nervous system as formulated by Werman (1966), were recently extended with criteria for neuromodulators, neuromediators, neurohumours etc. (Barchas et al., 1978; Bloom, 1979; Dismukes, 1979; Siggins, 1979). Arguments will be given for which points these two neuropeptides fulfil the criteria for being a neurotransmitter.

The wide-spread distribution of these two peptides throughout the central nervous system and their presence in synaptic structures is the fulfilment of a major criterium to establish a substance as a neurotransmitter. Equally important is the ability of these synapses to release their transmitter upon a depolarizing stimulus. Recent in vitro experiments (R.M. Buijs, unpubl. obs.) demonstrated a Ca^{2+} -dependent K^{+} stimulated release of vasopressin from the lateral septum and oxytocin release in the nucleus of the solitary tract. In addition, the central release of AVP upon a physiological stimulus was demonstrated by Cooper et al. (1979), who found that using a push-pull canule in the sheep septal region vasopressin release was negatively correlated with induced changes in body temperature. Receptor binding for vasopressin and oxytocin is still undemonstrable. However, often a good correlation is found between the central sites of action and the distribution of the areas where these peptidergic fibers appear to terminate

(Kovacs et al., 1979; Cooper et al., 1979; Buijs, 1978; Buijs and Swaab, 1979).

Finally the ability of neurohypophyseal peptides to change membrane potentials was demonstrated by Morris et al. (1980), who found an inhibition of glutamate induced or spontaneous firing after iontophoretically applied oxytocin to brain stem neurons in a region that is densely innervated by oxytocin containing fibers, the nucleus of the solitary tract.

It will thus be clear that these two neuropeptides are very good candidates for a function in the central nervous system as neurotransmitters. Although not all criteria are fully met, the arguments for such a role for the much longer studied aminergic or aminoacid neurotransmitters are often not better.

The ubiquitous presence of these peptides throughout the animal kingdom, even in insects, molecules closely related to neurophysins have been demonstrated (Friedel et al., 1980), while the human brain is also densely innervated by these peptidergic fibers, suggests important central functions of vasopressin and oxytocin. However, at present the limited data available are mostly from studies in rats (de Wied and Bohus, 1979); it seems likely from the latter physiological or behavioral observations in combination with the anatomical data, that these peptides might be involved in the integration of (stressful) stimuli and influence important autonomic functions like the regulation of temperature (Cooper et al., 1979), bloodpressure (Bohus, 1980; le Moal et al., 1981) and water balance (Raichle and Grub, 1978). Dysfunctioning of these processes in older individuals seems to coincide with a change in the staining of the vasopressin cells in the aged human brain (E. Fliers, unpubl. obs.), suggesting that these peptides may be essential compounds for the functioning of the central nervous system in development, adulthood and aging.

REFERENCES

- Alonso, G., Assenmacher, I., The smooth endoplasmic reticulum in neurosecretory axons of the rat neurohypophysis. *Biol. Cell* 32 203-206 (1978)
- Barchas, J.D., Akil, H., Elliott, G.R., Holman, R.B., Watson, S.J.: Behavioral neurochemistry: Neuroregulators and behavioral states. *Science* 200, 964-973 (1978)
- Bloom, F.E., Contrasting principles of synaptic physiology: peptidergic and non-peptidergic neurons. In: K. Fuxe, T. Hökfelt, and R. Luft (eds.), *Central Regulation of the Endocrine System*, pp. 173-187, Plenum Press, New York (1979)
- Boer, G.J., Swaab, D.F., Buijs, R.M., Uylings, H.B.M., Velis, D.N., and Boer, K., Neuropeptides in rat brain development. In: P.S. McConnell, G.J. Boer, H.J. Romijn, N.E. van de Poll, and M.A. Corner (eds.), *Adaptive Capabilities of the Nervous System*, *Progress In Brain Research*, Vol. 53. Elsevier, Amsterdam (in press)(1980)
- Bohus, B., Effects of neuropeptides on adaptive autonomic processes. In: D. de Wied and P.A. van Keep (eds.), *Hormones and the Brain*, pp. 129-139, MTP Press, Lancaster (1980)
- Buijs, R.M., Intra- and extrahypothalamic vasopressin and oxytocin pathways in the rat. Pathways to the limbic system, medulla oblongata and spinal cord. *Cell Tiss. Res.* 192, 423-435 (1978)
- Buijs, R.M., Vasopressin and oxytocin innervation of the rat brain. A light- and electronmicroscopical study. Ph.D. thesis, Amsterdam (1980)
- Buijs, R.M., and Swaab, D.F., Immunoelectron microscopical demonstration of vasopressin and oxytocin containing synapses in the limbic system of the rat. *Cell Tiss. Res.* 204, 355-365 (1980)
- Buijs, R.M., Swaab, D.F., Dogterom, J., and van Leeuwen, F.W., Intra- and extrahypothalamic vasopressin and oxytocin pathways

- in the rat. *Cell Tiss. Res.* 186, 423-433 (1978)
- Cooper, K.E., Kasting, N.W., Lederis, K., and Veale, W.L., Evidence supporting a role for endogenous vasopressin in natural suppression of fever in sheep. *J. Physiol.* 295, 33-45 (1979)
- Cushing, H., Paper relating to the pituitary body, hypothalamus and parasympathetic nervous system. C.C. Thomas (ed.), Springfield, Ill. U.S.A. (1932)
- Dismukes, R.K., New concepts of molecular communication among neurons. *Behav. Brain Sci.* 2, 409-448 (1979)
- Droz, B., Koenig, H.L., Giaumberardino, L.J., Axonal migration of protein and glycoprotein to nerve endings. I. Radioautographic analysis of the renewal of protein in nerve endings of chicken ciliary ganglions after intracerebral injection of ^3H -lysine. *Brain Res.* 60, 93-127 (1973)
- Friedel, T., Laughton, B.G., Andrew, R.D., A neurosecretory protein from *Locusta migratoria*. *Gen. Comp. Endocr.* 41, 487-498 (1980)
- Hoorneman, E.M.D., Buijs, R.M., Exohypothalamic vasopressinergic fibre pathways in the rat brain after lesioning of the supra-chiasmatic nucleus (submitted)
- Kovacs, G.L., Bohus, B., Versteeg, D.H.G., de Kloet, E.R., and de Wied, D., Effect of oxytocin and vasopressin on memory consolidation: sites of action and catecholaminergic correlates after local microinjection into limbic midbrain structures. *Brain Res.* 175, 303-314 (1979)
- Leeuwen, F.W. van, Swaab, D.F., and de Raay, C., Immunoelectron-microscopic localization of vasopressin in the rat supra-chiasmatic nucleus. *Cell Tiss. Res.* 193, 1-10 (1978)
- Le Moal, M., Koob, G.F., Koda, L.Y., Bloom, F.E., Manning, M., Sawyer, W.H., and Rivier, J., Vasopressor receptor antagonist prevents behavioural effects of vasopressin. *Nature* 291, 491-493 (1981)
- Morris, R., Salt, T.E., Sofroniew, M.V., and Hill, R.G., Actions of microiontophoretically applied oxytocin, and immunohistochemical localization of oxytocin, vasopressin and neurophysin

- in the rat caudal medulla. *Neurosci. Lett.* 18, 163-168 (1980)
- Pickel, V.M., Joh, T.H., Reis, D.J., Leeman, S.E., and Miller, R.J., Electron microscopic localization of substance P and enkephalin in axon terminals related to dendrites of catecholaminergic neurons. *Brain Res.* 160, 387-400 (1979)
- Raichle, M.E., Grubb, R.L., Regulation of brain water permeability by centrally released vasopressin. *Brain Res.* 143, 191-194 (1978)
- Siggins, G.R., Neurotransmitters and neuromodulators and their mediation by cyclic nucleotides. In: Y.H. Ehrlich, J. Volavka, L.G. Davis and E.G. Brungraber (eds.), *Modulators, Mediators and Specifiers in Brain Function*, pp. 41-64, Plenum Press, New York (1979)
- Sofroniew, M.V., and Weindl, A., Extrahypothalamic neurophysin containing perikarya, fiber pathways and fiber clusters in the rat brain. *Endocrinology* 102, 234-237 (1978a)
- Sofroniew, M.V., and Weindl, A., Projections from the parvocellular vasopressin and neurophysin containing neurons of the supra-chiasmatic nucleus. *Amer. J. Anat.* 153, 391-430 (1978b)
- Sternberger, L.A., *Immunocytochemistry*, New York: Prentice Hall, Englewood Cliffs (1974)
- Swaab, D.F., Pool, C.W., Specificity of oxytocin and vasopressin immunofluorescence. *J. Endocrinol.* 66, 263-272 (1975)
- Vandesande, F., Dierickx, K., and de Mey, J., Identification of the vasopressin neurophysin producing neurons of the rat supra-chiasmatic nuclei. *Cell Tiss. Res.* 156, 377-380 (1975)
- Wied, D. de, Long-term effect of vasopressin on the maintenance of a conditioned avoidance response in rats. *Nature* 232, 58-60 (1971)
- Wied, D. de, Bohus, B., Modulation of memory processes by neuropeptides of hypothalamic neurohypophyseal origin. In: M.A.B. Brazier (ed.), *Brain mechanisms in memory and learning: from the single neuron to man*, pp. 139-149, New York, Raven Press (1979)