Vasopressin and Oxytocin: Distribution and Putative Functions in the Brain

R.M. BUIJS, G.J. DE VRIES, F.W. VAN LEEUWEN and D.F. SWAAB

Netherlands Institute for Brain Research, IJdijk 28, 1095 KJ Amsterdam (The Netherlands)

LOCALIZATION AND ORIGIN OF VASOPRESSIN AND OXYTOCIN FIBRES IN THE RAT BRAIN

Vasopressin (AVP)- and oxytocin (OXT)- containing fibres have been demonstrated by immunocytochemistry in a large number of rat brain regions from the olfactory bulb down to the spinal cord (Swanson, 1977; Sofroniew and Weindl, 1978; Buijs, 1978). The densest networks occur in subcortical brain structures, though in the cortex and cerebellum scattered — mostly AVP — fibres are found (Buijs, 1980). Limbic system structures, such as the lateral septum, the lateral habenular nucleus and the amygdala, are particularly heavily innervated by AVP fibres, while OXT fibres are predominant in the brainstem and spinal cord (Buijs, 1978; Swanson and McKellar, 1979). These extrahypothalamic fibres, which are already present at foetal day 17 (Buijs et al., 1980), innervate the lateral septum and lateral habenular nucleus from day 10 in a sexual dimorphic way (De Vries et al., 1981), viz. in male rats a much denser AVP innervation was found. This sex difference appears to depend on the presence of androgens in early postnatal development. In adulthood the female innervation pattern of the lateral septum can be changed to that of the male by the administration of a large dose of testosterone, which demonstrates the plasticity of the innervation of this area (De Vries et al., unpublished). The presence of AVP and OXT innervation of the brain has been demonstrated in various other mammalian species; for example, human subcortical brain structures are extensively innervated by AVP and OXT fibres which can already be found in the spinal cord of human fetuses 19 weeks after conception (Swaab and Ter Borg, 1981). Van den Dungen et al. (1982) demonstrated an even more widespread vasotocin and isotocin fibre system in the trout brain, while also the brains of lampreys were found to be densely innervated by vasotocin fibres. Consequently, the presence of neurohypophysial hormone-containing extrahypothalamic fibres is not a phenomenon acquired by higher vertebrates but is also present in species which are phylogenetically considered to be older.

The extrahypothalamic AVP and OXT fibres in the rat brain were thought to be derived from the paraventricular (PVN) and possibly also the supraoptic nuclei (SON), and from the parvocellular, AVP-containing, suprachiasmatic nucleus (SCN). Originally, the anatomy of the AVP and OXT fibre pathways was established mainly by following the fibres in serial sections (Buijs et al., 1978; Buijs, 1978; Sofroniew and Weindl, 1978). However, lesioning the SCN showed that its AVP fibre system is much less widespread than these earlier studies had suggested. The AVP fibres from the SCN appear to reach the organum vasculosum of the lamina terminalis (OVLT), the dorsomedial hypothalamic nucleus and the periventricular

nucleus (Hoorneman and Buijs, 1982) (Fig. 1). Apart from the AVP projections from the SCN, only the AVP projection from the PVN towards the nucleus of the solitary tract (NTS) has been demonstrated convincingly by means of retrograde transport of horseradish peroxidase (HRP) in combination with the immunocytochemical detection of the peptide content of the cell bodies (Sofroniew and Schrell, 1980; Sawchenko and Swanson, 1982). In a study by De Vries and Buijs (1983), lesioning of the PVN indeed resulted in the disappearance of AVP fibres in several regions of the brainstem. Surprisingly, it was not followed by a diminution of AVP fibres in the various other regions where the PVN was thought to project to, e.g. the amygdala, ventral hippocampus, dorsal raphe nucleus, etc.

Fig. 1. AVP pathways and possible sites of termination ($\rightarrow$) in the rat brain projected in the sagittal plane. The pathways of the PVN (squares) are indicated by dashed-dotted lines ($\cdots\cdots\cdots$) and pathways of the SCN (triangles) are indicated by dotted lines ($\cdots\cdots\cdots$). The AVP cell groups found only after colchicine treatment are indicated by large black dots, while the pathways of the bed nucleus of the stria terminalis (BST) are indicated by dashed lines ($\cdots\cdots\cdots$). A question mark indicates that the source of the AVP innervation in this area is still unknown. Abbreviations: A, amygdala; DBB, diagonal band of Broca; DMH, dorsomedial nucleus of the hypothalamus; DVC, dorsal vagal complex; LC, locus coeruleus; LH, lateral habenula; LS, lateral septum; ME, median eminence; OVLT, organum vasculosum of the laminae terminalis; PVG, periventricular grey; PVS, periventricular nucleus; RD, dorsal raphe nucleus; VHi, ventral hippocampus; VTA, ventral tegmental area.

Recently Van Leeuwen and Caffé (1983) demonstrated in rats which had been treated with colchicine, numerous AVP cell bodies in other brain regions outside the hypothalamus, i.e. in the bed nucleus of the stria terminalis, the amygdala, locus coeruleus, and in the dorsomedial nucleus of the hypothalamus (Fig. 2). This opens the possibility that these nuclei are the source of a substantial part of the vasopressin innervation of the brain. After lesioning the cell groups in the bed nucleus of the stria terminalis the AVP fibre innervation of the lateral septum decreased drastically. Furthermore, small cell bodies in the bed nucleus of the stria terminalis were found to be labelled after injection of HRP and fluorescent tracers in the lateral septum. Thus it is likely that the bed nucleus is the origin of the AVP fibres in the lateral septum. The AVP neurones of the bed nucleus of the stria terminalis probably also project to the lateral habenular nucleus, the periventricular grey and locus coeruleus (De Vries and Buijs, 1983) (Fig. 1). Because lesions might also have caused the destruction of passing AVP fibres, ultimate proof for these projections can only be obtained after retrograde tracing in combination with the demonstration of AVP in the labelled neurone.

Nevertheless, a new picture emerges with respect to the origin of the AVP fibres, viz. that the PVN and SCN contribute only for a minor part to these extrahypothalamic fibres. The

Fig. 2. Transversal section of AVP-positive cell bodies in the rat brain, demonstrated in the region of the bed nucleus of the stria terminalis (BST). PVN is the most rostral part of the paraventricular nucleus. The arrows point to the AVP cells in the BST, which are only visible after colchicine treatment. Bar = 100 μ m.

origin of the major part must lie elsewhere, most probably in the AVP cell bodies that could be demonstrated only after colchicine treatment. Herewith AVP has lost one of its characteristics that had distinguished it so far from other peptides, in that its cell bodies were restricted to the hypothalamus and were readily visible without colchicine treatment. As it appears now, a combination of both cell types occurs, and the AVP cell bodies are much more widespread than has been assumed hitherto.

In contrast to AVP, practically all OXT fibres seem to be derived from the PVN, as lesioning of this nucleus results in an almost complete disappearance of the OXT innervation of the brain. Furthermore, in colchicine-treated rats no additional OXT neurones were detected, which reduces the probability of an alternative source. It appears, from its much more widespread sites of origin and terminal areas, that AVP is involved in the regulation of far more functions than OXT.

ELECTRON MICROSCOPIC LOCALIZATION

The assumption of Barry (1954) that neurosecretory fibres containing Gomori-positive material would terminate by means of "des synapses neurosecretoires" seemed to be plausi-

ble, as in many brain regions AVP- and OXT-containing fibres were found to form extensive ramification and perineuronal structures. Immunoelectron microscopy has been performed on regions in the limbic system and medulla that are densely innervated by AVP and OXT fibres, respectively. In these regions AVP and OXT have been localized within synapses mostly terminating on dendrites and less frequently on cell bodies. On no occasion were AVP- or OXT-containing structures found to be either pre- or postsynaptic to axonal processes (Buijs and Swaab, 1979; Voorn and Buijs, 1983). Therefore, results which suggest a "modulating" action of AVP on monoamine metabolism in terminal regions of monoaminergic and AVP fibres (Versteeg et al., 1979; Kovács et al., 1979) may be explained via an indirect mechanism and not via a direct presynaptic action of AVP on the monoamine-containing axon or terminal. In the same way, the absence of presynaptic terminals on AVP- and OXT-containing fibres suggests that presynaptic modulation of AVP and/or OXT release is not widespread, at least not in the areas investigated.

Although, for the visualization of these structures, it is necessary to use Triton X-100, which hampers the preservation of ultrastructure of the tissue, these synapses were found to contain clear vesicles and sometimes also 80–100 nm dense core vesicles (Buijs and Swaab, 1979). The presence of these dense core vesicles in extrahypothalamic brain regions suggests that the AVP and OXT fibres in these areas are derived from different neurones than those projecting to the neural lobe, where larger (140 nm) vesicles are found (Van Leeuwen et al., 1977). The presence of small vesicles might indicate a different processing mechanism for these peptides from that in the hypothalamo-neurohypophyseal system. The fact that, in the brainstem, immunoreactive neurophysin is present predominantly as a high molecular weight precursor in contrast to the precursors found in the hypothalamo-neurohypophyseal system (Sinding et al., 1982) supports this idea. Another possibility is that the release mechanism of these peptides in the brain is different from that in the pituitary, resulting in many more clear vesicles and fewer (and smaller) dense core vesicles.

In the human and in the trout and lamprey brain, the extrahypothalamic neurohypophyseal hormone fibres form similar ramifications and boutons similar to those found in the rat, which suggests that these fibres also terminate synaptically, and indeed such synaptic terminations have recently been demonstrated in the human brain (Kerkhoven and Buijs, unpublished). Thus there seems little doubt that these neurohypophyseal peptides influence neuronal structures via synaptic terminals in a wide variety of species. However, although the synaptic localization of AVP and OXT is established it remains an open question whether these peptides or other, unknown substances are released from these synapses.

CENTRAL RELEASE OF AVP AND OXT

To demonstrate that AVP- and OXT-containing synapses are able to release their peptide content upon depolarization, an *in vitro* release procedure was set up (Buijs and Van Heerikhuizen, 1982). For this purpose various brain regions and the neurointermediate lobe were selected. Using high potassium or veratridine as the depolarizing stimulus, release from the septal area and NTS (areas where AVP and OXT fibres terminate) was compared with the release of these peptides from the neural lobe.

A calcium-dependent potassium- or veratridine-stimulated release of AVP and/or OXT could be demonstrated only for the brain regions where these fibres terminate synaptically on neuronal structures. The veratridine-stimulated release of OXT from the NTS ($6.1\% \pm 0.9\%$ of the tissue stores in 10 min) or the neural lobe ($7.7\% \pm 0.6\%$ in 10 min) is more pronounced

than that of AVP from the septum ($3.6\% \pm 0.7\%$ in 10 min) or neural lobe ($1.4\% \pm 0.1\%$ in 10 min; values $\pm$ S.E.M.) (Buijs and Van Heerikhuize, 1982). This difference in sensitivity towards veratridine depolarization might point to different membrane properties for AVP and OXT neurones and a different release mechanism. As the AVP and OXT fibres between the PVN and SON are varicose and fail to evince synaptic specializations, this area was selected to study whether these peptides could also be released non-synaptically.

No potassium-stimulated release of AVP or OXT could be detected from these areas in spite of the large amounts of peptide. Taken together, the results suggest that AVP and OXT are released in the central nervous system and that this release occurs from synaptic terminals. That this release will also occur under physiological circumstances is demonstrated, for instance, by the experiments of Cooper et al. (1979), who found — by perfusion of the sheep septal area with a push-pull cannula — a release of AVP which was negatively correlated with a rise in body temperature.

Although research on the central action of AVP and OXT has gained momentum only recently, many pertinent neurotransmitter criteria have already been fulfilled. These criteria have been set up in relation to the action of acetylcholine on the neuromuscular junction. However, even acetylcholine cannot at present meet all neurotransmitter criteria in the brain fully satisfactorily.

THE POSSIBLE FUNCTIONS OF AVP AND OXT IN THE CENTRAL NERVOUS SYSTEM

Since the observation of Cushing (1932) that an injection of a posterior pituitary extract resulted in a drop in body temperature and in vasodilatation, various other putative central functions of AVP and OXT have been added. Recently the influence of AVP on thermoregulation (Cooper et al., 1979) and the involvement of AVP and OXT in the regulation of other autonomic functions has been documented in much more detail, both anatomically and physiologically (Bohus, 1980; Pittman et al., 1982; Swanson and Sawchenko, 1980; Sawchenko and Swanson, 1982). In addition, these peptides might be involved in learning processes (see De Wied, this volume). The question arises as to whether these various putative functions of AVP and OXT can be linked to what we have learned so far about the anatomical properties of peptidergic systems in the central nervous system.

From the present anatomical details a picture can be drawn of an extensive innervation of many areas in the CNS by AVP- and/or OXT-containing fibres. Moreover, retro- and anterograde tracing studies have revealed that most of these areas project directly to the PVN and SON. Depending on whether the PVN projects in turn with OXT or AVP fibres to such an area, a direct bidirectional pathway is established. The NTS and the dorsal nucleus of the vagus seem to fit this description. For example, it has been demonstrated that the PVN projects at least with AVP-, OXT-, enkephalin- and somatostatin-containing fibres to the dorsal vagus complex (Sawchenko and Swanson, 1982), while extensive projections from the NTS and dorsal nucleus of the vagus to the PVN have been described (Ricardo and Koh, 1978). The majority of the latter input to the PVN seems to be to the parvocellular part. As axons of the small medial cells of the PVN terminate on the dendrites of the larger lateral cells (Van den Pol, 1982), the incoming information on that part of the nucleus can be relayed throughout the PVN, thus enabling a direct feedback to the whole PVN. However, whether those neurones that project to the PVN are indeed also innervated by PVN fibres remains to be established. Anyhow, by this anatomical relationship the PVN seems to be able to modulate afferent and

efferent autonomic information (cf. Swanson and Sawchenko, 1980). The role of AVP and OXT in the connection between the PVN and the hindbrain will probably lie in the regulation of processes in which these peptides are also involved in the periphery — such as the control of blood pressure and lactation. The fact that AVP concentrations are affected in brainstem and neural lobe in spontaneously hypertensive rats (Mohring et al., 1980), and that both AVP and OXT influence blood pressure, especially in caudal brain regions (Bohus, 1980; Pittman, 1982; Matsuguchi et al., 1982), supports this theory.

This coupling of central and peripheral action of AVP and OXT seems to be a general phenomenon of hypothalamic peptides (see Swaab, 1982). An example of such central and peripheral coupling might be found in the action of AVP in limbic brain areas such as the lateral septum and the amygdala. Both areas are densely innervated by AVP, fibres and project to the PVN and/or SON (Tribollet and Dreifuss, 1981; Silverman et al., 1981; Pittman et al., 1981a, b; Poulain et al., 1981), but it awaits elucidation whether the neurones that project to the PVN and SON are innervated by AVP fibres. The demonstration of a simultaneous AVP release following a hypertonic saline injection both in the periphery and in the septum and amygdala (Pittman, 1982) supports the idea that central and peripheral release of AVP are coupled. The possibility exists that, via the connections from the septum to the PVN and SON, central AVP might influence release of AVP into the periphery. Another possibility is that the simultaneous central and peripheral release will occur upon a specific stimulus and result in the initiation of processes, both centrally and peripherally, that help the animal adapt to this stimulus. The importance of such a coupled action might be found in the effect of AVP in temperature control. AVP release in the septal region of sheep measured by push-pull perfusion correlated negatively with a change in body temperature. That is, the release of AVP increases when body temperature falls, while by raising body temperature release of AVP decreases (Cooper et al., 1979; Kasting et al., 1982). The conclusion that this septal release has functional significance is warranted by the addition of AVP to the perfusate, which resulted in a drop in body temperature. In separate experiments it was demonstrated that peripheral AVP levels are enhanced when body temperature is elevated (Cooper et al., 1979). Thus AVP might exert an antipyretic effect in the CNS, while in the periphery it prevents the loss of water via the kidney to prevent dehydration by the perspiration induced by a rise in temperature. Thus central and peripheral actions of AVP may be coupled. Whether this will be a general rule for AVP and OXT or even for all hypothalamic releasing hormones will certainly be a point of future investigation.

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