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Vasopressin Localization and Putative Functions in the Brain

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1. Introduction

Vasopressin (VP) and oxytocin (OX) have long been known to be synthesized in the two nuclei of the hypothalamus, the paraventricular nucleus (PVN) and supraoptic nucleus (SON) and secreted into the circulation in the neural lobe of the pituitary (see Chapter 1, this volume). As a result of this origin from the hypothalamus-neurohypophyseal system (HNS), these two neuropeptides were known as hypothalamic hormones; it is not so long ago that, through the pioneering work of De Wied (1965, 1971), the notion came to be accepted that these peptides might affect the central nervous system (CNS) as well (see Chapter 14, this volume).

In retrospect, it can be concluded that morphologically the first indications for a possible central function of VP and OX were found as early as the 1950s. Ernst Scharer was not only the first to formulate the concept of neurosecretion (Scharer and Scharer, 1937) but was also the first to demonstrate the existence of extrahypothalamic Gomori-positive pathways (Scharer, 1951). Legait and Legait (1957, 1958) found considerable evidence for similar pathways in many vertebrates. But finally, morphologically, a great deal of evidence was offered for a central role of neurohypophyseal peptides, particularly in work done by Barry (1958, 1961). Barry even suggested that the Gomori-positive substance, which he thought originated from the PVN, would be able to influence neuronal structures by means of *des synapses neurosécrétoires* (Barry, 1954). This was the same year in which Eccles *et al.* (1954) accepted and helped advance the concept of chemical transmission, a concept raised in the 1930s by Dale (1935). Barry *et al.* (1958)

and his colleagues, Legait and Legait (1958), even succeeded in demonstrating that VP and OX biological activity was present in those extrahypothalamic brain regions in which Gomori-positive fibers were found. Still, it was more than 20 years later that immunocytochemical (Swanson, 1977; Brownfield and Kozlowski, 1977; Buijs, 1978; Sofroniew and Weindl, 1978) and radioimmunoassay (RIA) techniques (Dogterom and Buijs, 1980) permitted the confirmation and extension of these original observations. Thus, the notion became accepted that neurohypophysial hormones may also be functioning as putative neurotransmitters in the CNS.

This chapter presents a brief description of the widespread occurrence of VP neurons in the rodent brain, by far exceeding that of its OX counterparts. In addition, an attempt is made to describe, on the basis of its origin, possible functions of VP in the various parts of the CNS (see also Chapter 10, this volume).

2. *Vasopressin: From Hormone to Neurotransmitter*

The possibility of raising antibodies to VP and neurophysin (NP), part of the VP precursor molecule (see Chapter 4, this volume), and of using these antibodies in immunocytochemical techniques was first used to clarify whether VP is present in cell bodies in both PVN and SON and how these peptides were distributed (Swaab, *et al.*, 1975a; Vandesande and Dierickx, 1975). Later, a detailed description of the number of VP neurons in the PVN and SON and in the hypothalamic islets containing VP and OX neurons became available (Swaab *et al.*, 1975a; Fisher *et al.*, 1979; Kelly and Swanson, 1980; Rhodes *et al.*, 1981). In addition, these techniques were used to demonstrate the "one cell-one hormone" concept, i.e., that VP neurons do not contain OX (Swaab and Pool, 1975; Vandesande and Dierickx, 1975; Van Leeuwen and Swaab, 1977). The application of these immunocytochemical techniques on the hypothalamus produced an unexpected finding: the demonstration of NP-containing (Vandesande *et al.*, 1974) and VP-containing neurons in the suprachiasmatic nucleus (SCN) (Swaab and Pool, 1975; Swaab *et al.*, 1975; Vandesande *et al.*, 1975). Recently, other sites of VP synthesis were revealed after pretreatment of a rat with colchicine: in the cell bodies of the bed nucleus of the stria terminalis (BNST), dorsomedial hypothalamus, medial amygdala, and locus coeruleus (Van Leeuwen and Caffé, 1983; Caffé and Van Leeuwen, 1983).

In search of a morphological explanation of the results of De Wied and his co-workers, Van Wimersma Greidanus *et al.* (1975, 1976), who demonstrated a central effect of VP on avoidance behavior, the possibility that VP is released directly into the cerebrospinal fluid (CSF) was investigated (Dogterom *et al.*, 1977). The assumption that a direct release of neurohypophysial hormones into the CSF could exist was based on the results of Vigh *et al.* (1967), Vigh-Teichman *et al.* (1970), and Dierickx (1962). These investigators demonstrated that in lower vertebrates the cell bodies of the PVN or its homologue, the magnocellular preoptic nucleus, had extensive contacts with the CSF. In a study conducted by Van

FIGURE 1. Transverse section through the most rostral part of the preoptic nucleus in the trout brain stained for isotocin. (A) Overview of the isotocin-containing parvocellular part of this nucleus. Note the processes of the isotocin neurons that make contact with the ventricle (V) (arrows). Bar: 20 μ m. (B) A detail of A showing more clearly the cellular process contacting the ventricle (arrows). Bar: 5 μ m. (From Van den Dungen *et al.*, 1982.)

den Dungen *et al.* (1982), this was confirmed by isotocin immunocytochemical staining (Fig. 1). No morphological evidence for such a mechanism could be obtained in the rat median eminence (Buijs, 1978), but several other papers have appeared that provide evidence for the penetration of the ependyma by VP fibers in the region of the PVN or in the region of the fourth ventricle (see Castel *et al.*, 1984). However, biochemical evidence for a direct release of VP from VP terminals into the CSF is lacking.

Improvement of the immunocytochemical techniques (Brownfield and Kozlowski, 1977; Swanson, 1977; Buijs, 1978; Buijs *et al.*, 1978, 1980; Sofroniew and Weindl, 1978*a,b*; Buijs and Swaab, 1979; Sofroniew, 1982) immediately showed the existence of intraparenchymal VP pathways. Extensive innervation of the rat brain was revealed by NP- or by VP- and OX-containing fibers. Initially, NP pathways from the PVN toward the lower brain stem were demonstrated (Swanson, 1977). This was followed by the demonstration of either NP (Brownfield and Kozlowski, 1977; Sofroniew and Weindl, 1978*a,b*) or VP fibers (Buijs, 1978; Buijs *et al.*, 1978) in many limbic structures in the rat brain. In rapid succession, a number of papers appeared in which the localization of either NP or VP fibers was described (Petter *et al.*, 1982; Nilaver *et al.*, 1980; Epstein *et al.*, 1983). The most detailed description to date is that by De Vries *et al.* (1985); these data are summarized in Table I. For a detailed description of VP fibers in the forebrain, the reader is referred to this paper and, concerning the description of the distribution of VP fibers in the spinal cord, the paper by Swanson and McKellar (1979) remains unequaled.

The central VP innervation therefore suggested that VP is able to reach neuronal structures by other routes than via the general circulation or the CSF. Indeed, the presence of extensive fiber arborizations and perineuronal structures suggested that these fibers actually terminate in many such areas. Immunoelectron microscopy employed on the lateral septum, lateral habenular nucleus, amygdala, and nucleus tractus solitarius (NTS) showed that VP fibers terminate synaptically on other neuronal structures (Buijs and Swaab, 1979; Voorn and Buijs, 1981) (Fig. 2). Such terminals were frequently found on dendrites and occasionally contained stained vesicles of ± 90 nm. Since the same preembedding staining procedure in the neural lobe yields ± 140 -nm immunopositive vesicles (Buijs and Swaab, 1979), other classes of neurons must be involved in providing the extra hypothalamic innervation than those projecting toward the neural lobe. Immunoelectron microscopy with NP-antiserum has shown, in the lower brain stem, a few immunopositive terminals with dense-core vesicles of approximately 140 nm (Petter *et al.*, 1982). Thus, they probably belong either to the few PVN neurons that project both to the neural lobe and to the lower brain stem (Swanson and Kuypers, 1980) or to the SON neurons that project dorsally (Hawthorn *et al.*, 1985).

With the demonstration of VP in synaptic terminals, it became most likely that VP could be released synaptically, directly onto its target cell. It was already evident that, in rat, VP infusions were most active in behavioral (Van Wimersma Greidanus *et al.*, 1976; Kovács *et al.*, 1979) or physiological processes (Cooper *et al.*, 1979) in those brain regions that contain a dense VP innervation as well. Naturally, an answer was yet to be provided for the question of whether VP contain-

TABLE I
Distribution of Vasopressin Fibers in the Rat Brain^a

	Density of fibers ^b	Disappearance after castration	Brain regions that innervate the PVN and/or SON ^c
Telencephalon			
Olfactory nucleus	+		
Cingulate cortex	+		
Prefrontal cortex	+		
Olfactory tubercle	++		
Diagonal band of Broca	+++	*	**
Rostral lateral septum	++	*	**
Caudal lateral septum	+++	*	**
Organum vasculosum of the lamina terminalis	++		**
Subfornical organ	+		**
Diencephalon			
Anterior amygdaloid area	++	*	
Lateral amygdaloid nucleus	++	*	
Medial amygdaloid nucleus	+++	*	
Basal nucleus of Meynert	++	*	**
Medial preoptic area	+++		
Dorsomedial hypothalamic nucleus	+++		
Arcuate nucleus	+		
Premammillary nucleus	+		
Paraventricular thalamic nucleus	+++		
Rhomboid thalamic nucleus	+		
Mediodorsal thalamic nucleus	++	*	
Lateral habenular nucleus	+++	*	
Mesencephalon-Metencephalon			
Ventral hippocampus (stratum oriens, stratum moleculare, subiculum)	++	*	**
Entorhinal cortex	+	*	**
Pineal gland	+		
Supramammillary nucleus	++		
Ventral tegmental area	++	±*	
Substantia nigra pars compacta	+		
Central gray	++	*	
Dorsal raphe nucleus	++	*	
Median raphe nucleus	++	*	
Locus coeruleus	+	*	
Dorsal parabrachial nucleus	++	*	**
Myelencephalon			
Raphe magnus	+		
Nucleus of the solitary tract	+		**
Dorsal nucleus of the vagus	+		
Nucleus ambiguus A1 area	+		**
Intermediate lateral column spinal cord	+		**

^aData compiled from De Vries *et al.* (1985).

^bVP density of brain areas: + + +, high density; ++, moderate density; +, low density.

^cSee Section 6 for discussion; see also Chapter 1, this volume.

FIGURE 2. Electron micrograph of a vasopressin (VP)-positive terminal forming a synapse (arrow) with an unlabeled dendrite in the lateral habenular nucleus. Bar: 0.25 μ m (From Buijs and Swaab, 1979.)

ing fibers terminating in the CNS would be able to release VP or whether another unknown substance was released instead. First, Hawthorn *et al.* (1984) demonstrated that synaptic vesicle fractions of several extrahypothalamic brain regions were very rich in VP. Second, evidence for central release of VP was provided by Cooper *et al.* (1979), who demonstrated, in push-pull perfusates of the septal region, that VP release was correlated negatively with body temperature. *In vitro* depolarization studies using high K^+ levels supported a synaptic release as well. A marked increase of VP release from septal slices was shown upon this stimulation (Buijs and Van Heerikhuize, 1982) (Fig. 3). When similar slices from the region between the PVN and SON were exposed to the same K^+ depolarization,

FIGURE 3. Fractional release of vasopressin (VP) from septal slices in an *in vitro* system (A), and from the region between the paraventricular (PVN) and supraoptic nuclei (SON) (B). The initial release during the dissection of the material is indicated in the hatched column. The notation $-Ca^{2+}$ indicates no calcium; $+K^{+}$ indicates high potassium (56 mM); Ver indicates veratridine (K^{+} -agonist) in the medium. The detection limit of the assay is indicated with the stippled line. The total sum of the amounts present in the tissue after the experiment and of the peptide released are indicated by the 100% volume. (A) Veratridine-stimulated VP release is not different from potassium-stimulated release. The 100% value indicates the amount from 3 septa. (B) Neither potassium nor veratridine stimulated release could be observed for VP from this brain region, where VP fibers do not exhibit synaptic termination. The 100% value indicates the amount from three PVN/SON regions.

no increased release could be observed. Since VP-containing fibers between the PVN and SON do not exhibit synaptic specializations, these results indicate that VP can be released in regions where these fibers terminate synaptically. Finally, another experiment in which synaptic VP release is demonstrated was reported by Pittman *et al.* (1984), who stimulated the PVN electrically and found increased VP levels in perfusates of the subarachnoid space of the spinal cord *in vivo*. It therefore seems logical to conclude that VP will be released upon a specific stimulus and will act only in those areas in which synaptic specializations are present. It remains uncertain, however, whether VP will only have a physiological action in those brain regions in which VP terminals are present and not in other more distant areas. Since, we do not know whether any reuptake mechanisms for VP exist, and since central degradation of VP is slow (Burbach *et al.*, 1984), it may

well be that VP diffuses away from the site at which it is released and can thus reach receptors located elsewhere. In addition, it has been demonstrated that enzymatic breakdown of VP results in molecules with a behavioral VP activity much higher than with the original VP (Burbach *et al.*, 1983; see also Chapter 13, this volume). Thus, these VP fragments may also reach structures other than those in the immediate vicinity of the VP synapse.

Because the synaptic release of VP by K^+ depolarization is Ca^{2+} dependent (Buijs and Van Heerikhuize, 1982), it can be stated that in this respect VP acts as a neurotransmitter. In addition, several reports have appeared showing that VP affects neuronal firing in the lateral septum, hippocampus, and other brain regions (Zerihun and Harris, 1981; Mühlethaler *et al.*, 1982; Joëls and Urban, 1982, 1984; Mizuno *et al.*, 1984; See also Chapter 6, this volume) and that in many of these brain regions VP binding occurs (Baskin *et al.*, 1983; Biegon *et al.*, 1984; Van Leeuwen and Wolters, 1983; De Kloet *et al.*, 1985; see also Chapter 12, this volume). It may therefore be concluded that this peptide will act as a neurotransmitter in these brain regions (Buijs, 1983). With a neurotransmitter role of VP being basically established, it has become necessary to determine the functional meaning of this transmitter as well as how and where this function can be executed.

3. *Origin of the Vasopressinergic Fibers in the Brain*

Since VP can act as a neurotransmitter in several areas of the CNS, and is only released in areas in which its fibers terminate synaptically or in neurohemal contact zones, it has become important to determine the origin of the central VP innervation, the distribution of VP efferents, and by what stimuli VP release can be induced.

3.1. *Paraventricular and Supraoptic Nucleus*

Developmental studies have contributed to the knowledge of the origin of extrahypothalamic fibers. Before the SCN had developed, VP (and OX) fibers could be visualized in rostral and caudal brain regions, suggesting an origin in the PVN or SON. The early appearance of VP fibers in the olfactory area, even before the PVN was visible with immunocytochemical techniques, suggests that these fibers are at least partly derived from the SON (Buijs *et al.*, 1980). However, more information was obtained in elegant studies combining immunocytochemistry and anatomical techniques (Sawchenko and Swanson, 1982; Swanson and Kuypers, 1980; Sofroniew and Schrell, 1981). Using a combination of immunocytochemistry and labeling with retrograde tracers, they showed that at least a part of the VP- and OX-containing fibers in the medulla was derived from the PVN (Fig. 4). Recent lesion studies of the SON and PVN followed by RIA measurements of VP indicate that, not only the PVN, but also the SON may contribute to central VP innervation (Hawthorn *et al.*, 1985).

FIGURE 4. Diagram of the vasopressin (VP) pathways from the paraventricular nucleus (PVN). Question mark indicates that possibly also the supraoptic nucleus (SON) contributes to the extrahypothalamic pathways. Vasopressin fibers reach the olfactory nucleus (ON); pineal (P), dorsal parabrachial nucleus (DPB), dorsal vagal complex (DVC), lateral reticular nucleus (LRT), and nucleus ambiguus (Amb). The neurosecretory pathways from PVN and SON to the pituitary (see Chapter 1, this volume) and of the PVN to the median eminence (ME); are indicated as well. (See Chapter 11, this volume.)

3.2. *Suprachiasmatic Nucleus*

Until the studies cited in Section 3.1, the origin of many extrahypothalamic fibers was only indicated by nonanatomical methods, such as tracing these fibers in serial sections (Buijs, 1978; Buijs *et al.*, 1978) or by the assumption that fibers with a fine varicose appearance were derived from the SCN (Sofroniew and Weindl, 1978). The diameter of the VP-immunopositive vesicles of the SCN cells and of their fibers (~ 95 nm) (Van Leeuwen *et al.*, 1978) is close to the sizes found extrahypothalamically (see Section 2). This led to the suggestion that the SCN was the major source of VP fibers in the CNS ranging from the septum (Buijs, 1978; Sofroniew and Weindl, 1979; De Vries *et al.*, 1981) to the nucleus of the solitary tract in the brain stem (Sofroniew and Weindl, 1978*b*). Tracing fibers in serial sections and fiber morphology are not reliable methods for determining the source of extrahypothalamic fibers.

Evidence that the SCN was not the major source for VP in the brain was derived from experiments lesioning this nucleus and finding a decrease of VP innervation in only a few brain regions (Hoorneman and Buijs, 1982) (Fig. 5). The results from the lesioning study have corresponded well with anterograde tracing studies reported by Berk and Finkelstein (1981) and Stephen *et al.* (1981).

3.3. *Bed Nucleus of the Stria Terminalis*

One of the brain regions in which VP fibers are most numerous is the lateral septum. A series of lesioning and tracing experiments was conducted in order to

FIGURE 5. Schematic representation of the vasopressin (VP) pathways from the supraoptic nucleus (SCN). Vasopressin containing fibers from this nucleus reach the organum vasculosum of the lamina terminalis (OVLT), the periventricular thalamic nucleus (PV), the dorsomedial hypothalamic nucleus (DMH), and the periventricular nucleus of the hypothalamus (Pe).

determine the origin of these VP fibers (De Vries and Buijs, 1983). Lesioning of the PVN did not result in any diminution of VP staining of the lateral septum. Lesioning the PVN plus the hypothalamic magnocellular neurons in the accessory and supraoptic nuclei had the same result. Moreover, retrograde tracers in the lateral septum showed no labeled cell bodies in the PVN or in the SON, but retrograde-filled cell bodies were found in the BNST. In the same period, Van Leeuwen and Caffé (1983) demonstrated VP containing cells in this area after having used an *in vivo* colchicine treatment. Lesioning this main region did indeed result in a severe diminishment of VP fibers in the lateral septum, but also in the diagonal band of Broca and the lateral habenular nucleus (De Vries and Buijs, 1983). This finding suggests that VP fibers in these brain regions are derived from the BNST (Fig. 6).

3.4. Other Cell Groups

Apart from the BNST, a number of other cell groups were found to contain VP after colchicine treatment: a few cells in the dorsomedial hypothalamus and a cell group in the medial amygdala and the locus coeruleus (Caffé and Van Leeuwen, 1983; Sofroniew, 1985). In the latter cell group, VP appeared to be colocalized with noradrenalin (Caffé *et al.*, 1985). Retrograde tracing studies combined with immunocytochemistry (Caffé *et al.*, 1986) indicate that the neurons in the medial amygdala are the major source of the VP fibers in the ventral hippocampus. All in all, these observations make it possible to get a more complete picture of the extrahypothalamic VP fibers and their origin (Figs. 5 and 6), although several significant gaps remain to be filled in our knowledge of the anatomy of VP systems (Fig. 6).

FIGURE 6. Scheme of the vasopressin (VP) pathways from the bed nucleus of the stria terminalis (BST) and from the medial amygdaloid nucleus (MA). Filled circles indicate the vasopressinergic cell bodies that are under the influence of gonadal hormones, while open circles indicate vasopressin cell bodies in the dorsomedial hypothalamic nucleus (DMH) and locus coeruleus (LC) of which the projections are unknown (question marks). All cell bodies indicated are only visible after *in vivo* colchicine pre-treatment of the rat. Cell bodies of the BST project to the lateral septum (LS), diagonal band of Broca (DBB), olfactory tubercle (Tu), lateral habenular nucleus (LH), central grey (CG), dorsal Raphe nucleus (DR), and locus coeruleus (LC). Cell bodies of the medial amygdaloid nucleus project to the ventral hippocampus (Hip) and to the lateral septum (LS), and most probably serve as interneurons for the innervation of the medial amygdala itself. The source of the innervation of the mediodorsal thalamic nucleus (MD) and of the ventral tegmental area (VTA) is unclear.

4. Sexually Dimorphic Vasopressin Innervation of the Brain

While studying the development of the extrahypothalamic fiber system, De Vries *et al.* (1981) detected the presence of a sexually dimorphic innervation pattern of VP fibers in the lateral septum (Fig. 7). From postnatal day 12 onward, a male rat was found to have a much denser VP innervation in the lateral septum than that of a female rat. A series of castration and testosterone supplementation experiments, followed by the examination of VP fiber density in the lateral septum on day 26 postnatally, showed that the development of the sex difference depends on the presence of androgens in the male rat (De Vries *et al.*, 1983). Moreover, the presence of gonadal hormones, either testosterone or estradiol, appeared to be crucial to the maintenance of the VP fiber innervation of, e.g., the lateral septum (De Vries *et al.*, 1986). Gonadectomy of adult male or female rats resulted in a gradual decrease in VP fiber density over a period of 15 weeks to a point where few fibers could be found (De Vries *et al.*, 1984) (Fig. 8). The sexual dimorphism was also visible in the BNST, where the number of VP cells found in males exceeded that found in females (Van Leeuwen *et al.*, 1985). Moreover, upon castration, the number of VP cells decreased, a condition that could be reversed completely by testosterone substitution therapy. In all areas in which VP fibers are changing after gonadectomy, these fibers are derived from the BNST or from the medial amygdala. No effect of this hormonal treatment could be seen in the brain regions in which the VP fibers were derived from the SCN or PVN (De

FIGURE 7. Transverse sections of the lateral septum of the rat stained immunocytochemically for the presence of vasopressin (VP). Note that the density of the VP fiber network in the female (A) is lower than in the male rat (B). (From De Vries *et al.*, 1981.)

Vries *et al.*, 1985 (Fig. 9). These results indicate that apart from the possibility of dividing the extrahypothalamic pathways on the basis of their origin, it is also possible to divide them on the basis of their sensitivity to gonadal hormones (Figs. 6 and 9; Table I). The decrease in NP innervation of the lateral septum upon hypophysectomy (Yulis and Rodrigues, 1982) can most probably be attributed to the removal of gonadotropin, resulting in a decrease of testosterone levels. The morphology and origin of the various VP systems are rather well established; with this point of view in mind, it may be possible to make a few assumptions on the putative functions of VP in the brain.

5. Putative Functions of Vasopressin in the Central Nervous System

Vasopressin, or its ancestral peptide vasotocin, has been demonstrated in extrahypothalamic brain regions in all classes of vertebrates from the most prim-

FIGURE 8. Transverse sections of the area around the third ventricle (III) stained immunocytochemically for the presence of vasopressin (VP). The disappearance of the VP fiber network in the lateral habenular nucleus (LH) of a male rat is shown 15 weeks after castration (A), in contrast to a control rat (B). Note that this difference is absent in the paraventricular thalamic nucleus (PV). (From De Vries *et al.*, 1984.)

itive, the Agnathans (Van Dungen *et al.*, 1981), to mammals, including man (Fliers *et al.*, 1986). The fact that VP or related peptides are maintained throughout evolution supports their physiological significance in nervous system function. It seems clear that the diverse origin and widespread distribution of VP fibers indicates that VP is involved in the regulation of various functions in the CNS. Each of the sites in which VP is produced is influenced by different stimuli, thereby permitting a spatially controlled release of VP. Several long-term experiments have been carried out in order to determine whether a simultaneous release of VP into the general circulation and into central brain regions occurs using various physiological and nonphysiological stimuli (Robinson, 1983). Recently, Stark *et al.* (1984) reported that severe hypoxia in sheep led to a strong increase of VP in both CSF and plasma. The increase of VP in CSF was delayed in time and of a lesser magnitude than the increase in plasma VP. Induction of comparable VP levels in plasma by VP infusion failed to elevate CSF VP, suggesting that, at least under these conditions, no VP can reach the CSF from plasma. These data suggest that under extreme conditions such as hypoxia a

FIGURE 9. Schemes of the major areas in which vasopressin (VP) terminals were seen to disappear after castration. For abbreviations, see Fig. 6. VDB, nucleus of the vertical limb of the diagonal band; SNC, substantia nigra, pars compacta. The asterisk (*) indicates the cell bodies in the bed nucleus of the stria terminalis and in the medial amygdala of which the colchicine-induced VP staining disappears upon castration. (From De Vries *et al.*, 1985.)

simultaneous release of VP into the CNS and into the general circulation can occur.

In addition, several papers have appeared that describe changes in VP content in various brain regions after different experimental conditions (Epstein *et al.*, 1983; Doris and Bell, 1984; Zerbe and Palkovits, 1984), but often the results of these studies are difficult to interpret. Here, two studies are mentioned that claim a release of VP into the CNS by measuring a decline in VP levels in central brain regions where fiber terminals are found. One report provides data suggesting a release of VP from the septal area that is different from the release of VP from the amygdala under conditions of a passive-avoidance test (Laczi *et al.*, 1983). This is surprising, since the data presented in the present review suggest a homology between the BNST, septum, and medial amygdala. Another report elaborated on the effect of endotoxin-induced fever on the release of VP into the septal area and amygdala (Kasting and Martin, 1983). Both in the septal area and in the amygdala, a decrease in VP content was seen after endotoxin-induced fever. Both studies were done by groups that have established, by means of VP injection in the septal area, a role for this peptide in the process of passive-avoidance behavior (De Wied, 1983) and fever suppression (Kasting *et al.*, 1982; Banet and Wieland, 1985).

In order to describe putative functions for VP in central brain regions, this chapter takes into account the source of VP fibers, in distinguishing between the different VP systems of the brain.

5.1. *Paraventricular Nucleus*

The PVN supplies the largest contingent of VP and OX fibers, the latter predominating, in the more caudal regions of the brain, viz. brain stem and spinal cord (Buijs, 1978; Swanson and McKellar, 1979; Nilaver *et al.*, 1980) (Fig. 4). The VP projection to the A1 and A2 areas and to the intermediolateral column in the spinal cord has been the most studied of the PVN projections. It has been shown that especially A1 (noradrenergic) neurons densely innervate the magnocellular VP- and OX-containing neurons in the SON and PVN (Sawchenko and Swanson, 1981; Sladek and Zimmerman, 1982). Day and Renaud (1984) and Day *et al.* (1984) have shown by a combination of lesioning with 6-hydroxydopamine and stimulation of the A1 area that this noradrenergic innervation provides a stimulatory input for the VP-containing neurons of the SON and PVN, thereby promoting the neurohypophysial release of VP into the bloodstream. The innervation of A1 and A2 neurons by VP-containing fibers suggests that VP as a neurotransmitter can be involved, for example, in controlling the release of VP from the HNS into the general circulation. Further studies will be necessary to provide experimental support for this hypothesis. At present, however, data linking peripheral release of VP to central release under physiological conditions are often conflicting. Several reports have appeared showing a high peripheral release of VP with no effects on central VP, neither in the brain stem, in spinal cord regions, or in the CSF (see Mens *et al.*, 1980; Robinson, 1983).

As put forward by Swanson as early as 1977, it seems most likely that VP and OX in caudal brain stem and spinal cord are involved in the regulation of autonomic functions (Swanson, 1977). Such functions may be related to the function of VP as a hormone in the general circulation, since A1 and A2 areas are intimately involved in the regulation of blood pressure and the same is true of those regions of the spinal cord that are innervated by VP fibers. Several studies using injection or infusion techniques into the CSF or brain stem regions have already described effects of VP on blood pressure regulation (Bohus, 1983; Versteeg *et al.*, 1980; Matsuguchi *et al.*, 1982; Pittman *et al.*, 1982; Berecek *et al.*, 1983, 1984; Martin *et al.*, 1985; Pittman and Franklin, 1985). Another possible function of VP in the spinal cord can be inferred from the VP innervation of neurons that control the blood circulation of the kidney. Riphagen and Pittman (1985) showed that an intrathecal injection of VP into the lower thoracic region of the spinal cord induced a clear antidiuretic action due to a neuronal action on the kidney. This study and those in which VP was shown to have an effect on blood pressure are the first pieces of evidence for the hypothesis that VP as a central neurotransmitter may be involved in the regulation of the same functions influenced by VP as a hormone in the general circulation (see also Chapter 10, this volume).

5.2. *Suprachiasmatic Nucleus*

The circadian rhythms are synchronized mainly through the information conveyed to the SCN via the retinohypothalamic tract. Herein, the SCN, the

major endogenous pacemaker, functions as the major biological clock in synchronizing the circadian rhythms (Stephan and Zucker, 1972; Stephan and Nunez, 1977; Nunez and Casati, 1979; Moore, 1979). It seems only logical to assume that VP from the SCN is involved, one way or another, in this synchronizing function. The fact that a VP-deficient Brattleboro rat, which lacks VP in the SCN as well, seems to have a normal sleep-wake rhythm (Peterson *et al.*, 1980) does not necessarily lead to the conclusion that VP is of no importance to the circadian synchronization of certain behaviors. These mutant rats are likely to have compensatory systems that, at least partially, take over the function of VP. Since VP from the SCN reaches the organum vasculosum of the laminae terminalis (OVLT), the paraventricular nucleus of the thalamus and dorsomedial hypothalamic nucleus, it seems worthwhile to investigate what functions these areas are involved in and which of these functions are strongly influenced by circadian patterns. The OVLT seems to be involved, for example, in the regulation of drinking behavior (Thrasher *et al.*, 1982), and the dorsomedial hypothalamus in feeding behavior (Leibowitz, 1975; Crine, 1983). Both behaviors are closely related and linked to circadian rhythmicity. It seems possible, therefore, that VP in these regions influences these behaviors, a possibility that should be checked via local infusion of this peptide into these areas.

In the CSF, VP has a pronounced day-night rhythm that seems to be dependent on the activity of the SCN (Reppert *et al.*, 1981, 1982, 1985; Schwartz *et al.*, 1983). Södersten *et al.* (1983, 1985) have shown that VP injected into the CSF during low VP night levels inhibits lordosis behavior in ovariectomized female rats, while an antagonist injected during the day period enhances lordosis behavior. Their observations led them to the conclusion that VP inhibits lordosis behavior and to suggest that the SCN is involved in the regulation of this behavior.

5.3. *Bed Nucleus of the Stria Terminalis and Medial Amygdala*

These nuclei are discussed together because both are influenced by gonadal steroids and probably both project to the same areas (Holstege *et al.*, 1985). It has been shown that the presence of VP in the neurons of the BNST and medial amygdala is strongly influenced by gonadal hormones (Van Leeuwen *et al.*, 1985). In contrast to their fiber systems, the VP content of these nuclei only shows up clearly after colchicine treatment (Van Leeuwen and Caffè, 1983; Caffè and Van Leeuwen, 1983). The decreased VP content in the BNST and medial amygdala neurons (Van Leeuwen *et al.*, 1985), as well as in their processes into the various areas of the limbic system after castration (De Vries *et al.*, 1983, 1984, 1985) are most likely the result of a decreased VP synthesis in these VP neurons. Disappearance of VP immunoreactivity in a fiber after castration is probably the result of the imbalance between the rate of synthesis in the cell body and the release in the terminals. Since in colchicine-treated gonadectomized rats no VP could be demonstrated in the neurons of the BNST (Van Leeuwen *et al.*, 1985), it is likely that the synthesis of VP is greatly diminished. Such a view is supported by observations in connection with the luteinizing hormone releasing hormone (LHRH)

system. In male rats, castration leads to an increase of LHRH release into the portal blood (Eskay *et al.*, 1977), whereas the number of LHRH-immunoreactive cell bodies decreases (Shivers *et al.*, 1983). By contrast, castrated rats are treated with colchicine, this leads to an increase in the number of LHRH-immunoreactive cell bodies (Shivers *et al.*, 1983). Therefore, the failure to detect VP cell bodies in the BNST and medial amygdala in castrated rats pretreated with colchicine suggests that gonadal hormones are necessary to stimulate the VP synthesis in these neurons. It therefore seems probable that under conditions of low estrogen and testosterone levels the release of VP in the limbic brain regions innervated by these nuclei is diminished. The sexually dimorphic pattern of innervation and the fact that replacement of estrogen and testosterone restores the original pattern of VP innervation (in gonadectomized females and males respectively; De Vries *et al.*, 1984, 1985) suggest that VP in these limbic brain regions is involved in the regulation of sex-linked functions. This does not imply, however, that VP is involved in the regulation of reproductive behavior, for a number of other behaviors such as feeding, drinking, temperature regulation, and aggressive behavior is sexually dimorphic as well (Goy and McEwen, 1980; Beattey, 1984). Thus, the sexually dimorphic VP innervation may be involved in nonreproductive behavior-linked sexually dimorphic functions.

In order to gain a better insight into the possible physiological correlates of sexual dimorphism in VP innervation, the pattern of VP innervation was studied in animals which show dramatic changes in blood gonadal hormone levels under natural conditions. In the European hamster, it was found that in the period preceding hibernation, when testosterone levels are extremely low, the VP innervation in the sexually dimorphic areas also disappears. In contrast, during the spring period, when testosterone levels have risen again, the VP innervation is completely restored (Buijs *et al.*, 1986). Here, again, we see that in the process of hibernation, the functions that change are the same as those mentioned before: reproductive behavior, drinking, feeding, temperature, and blood pressure regulation, which implies that the changes in VP innervation are involved in these physiological changes. The drastic changes in testosterone levels, as they occur during hibernation and castration, have been shown to influence more transmitter systems than does the VP system alone.

6. *Coupling of Central and Peripheral Actions*

Another interesting phenomenon is the apparent reciprocal pattern of innervation between many regions that are densely innervated by VP fibers and those that project to the PVN and SON (Poulain *et al.*, 1981; Silverman *et al.*, 1981; Tribollet and Dreifuss, 1981; Tribollet *et al.*, 1985; Pittman *et al.*, 1981) (see Table I). For instance, small injections of tracer into the PVN or SON results in the retrograde labeling of neurons in the lateral septum, ventral hippocampus, and medial amygdala. Although it cannot be excluded that a portion of these retrogradely labeled neurons project only to the immediate vicinity of the PVN and SON and are labeled by the spreading of the tracer from the injection site, it seems clear that these areas provide afferents to the PVN and SON, where they

can terminate on the broad expanse of dendrites from these two nuclei (Van de Pol, 1980; see also Chapter 1, this volume). In addition, stimulation of the lateral septum or medial amygdala results in inhibition or excitation of VP- or OX-containing magnocellular neurons in the PVN and SON (Pittman *et al.*, 1981; Poulain *et al.*, 1981), while anterograde tracing showed that lateral septum neurons innervate VP-containing dendrites in the SON and PVN (Oldfield *et al.*, 1985). It needs to be established, however, by means of retrograde tracing from the PVN or SON combined with VP immunocytochemistry, whether these lateral septal neurons are also innervated by fibers containing VP. These observations may again imply that the central and peripheral actions of VP are coupled and that the central release of VP in some brain regions may influence the peripheral release of VP. In fact, several reports suggest such a coupling (Cooper *et al.*, 1979; Kasting *et al.*, 1981; Laczi *et al.*, 1983a,b), since following a stimulus, this may help the animal adapt properly to the changed environment.

The importance of such a coupling mechanism can be found in the effect of VP on temperature control. VP release into the septal region of sheep, measured by push-pull perfusion, correlates negatively with a change in body temperature. That is, the release of VP increases when body temperature falls, while raising the body temperature leads to a decreased release of VP (Cooper *et al.*, 1979; Kasting *et al.*, 1982). The conclusion that this septal release has some functional significance is warranted by the addition of VP to the perfusate, which results in a drop in body temperature. In separate experiments it was demonstrated that peripheral VP levels are enhanced when body temperature is elevated (Cooper *et al.*, 1979). Thus, VP might induce an antipyretic effect in the CNS, while in the periphery it prevents the loss of water via the kidney, to compensate for the increase in perspiration produced by a rise in temperature. Thus, central and peripheral actions of VP may be coupled.

Whether such a mechanism of mutual central and peripheral effects holds for all areas in which VP fibers terminate will be a point of future research. It also seems worthwhile to investigate the possible effects of VP on a behavior known to be related to one of the aforementioned brain regions (Table I) and at the same time can be related to known functions of VP in the periphery such as drinking behavior. However, one must bear in mind that the influence of VP on behavior may be affected by mechanisms other than those involved in, for example, central thermoregulation. VP in the picogram range influences passive avoidance behavior (Kovács *et al.*, 1980), while in the nanogram range it affects thermoregulation (Kasting *et al.*, 1982). In addition, Kovács and De Wied (1983) showed that behaviorally effective VP analogues are without any effect on thermoregulation. Since the amount of VP in local brain regions is quite low— ± 200 pg in the total septum (Dogterom *et al.*, 1978)—it seems that a massive release of VP is necessary for a physiological effect on thermoregulation, while passive-avoidance behavior could theoretically be influenced by a much lower release from the terminals. It is therefore surprising that in a behavioral situation such massive release is suggested to take place (Laczi *et al.*, 1983). Moreover, this release is reported to take place only in the septum and not in the amygdala (Laczi *et al.*, 1983a,b), while for endotoxin-induced VP release the loss of VP in these areas was correlated (Kasting and Martin, 1983).

Recently it was reported that rats that performed a large number of learning tasks very well during many behavioral sessions had a much denser VP innervation in the lateral septum than rats with a bad performance (Ermisch *et al.*, 1986). Since the density of VP innervation in the lateral septum is influenced by gonadal hormones, it would be interesting to see whether this better performance is related only to VP or if it can also be correlated with testosterone levels in the general circulation.

Although we seem to know quite a lot about the morphology of the various VP systems in the brain, we are only at the beginning of understanding the physiological meaning of the VP innervation in the many brain regions that are a target for this peptide.

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