Vasopressin neurotransmission: involvement in temperature regulation and stress response

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ABSTRACT

For the central functions of vasopressin (VP) three different systems can be distinguished, depending on the origin of this neuropeptide. In the present paper we propose that 1) VP originating in the paraventricular nucleus may be involved principally in gating autonomous information to and from the central nervous system; 2) VP originating from the bed nucleus of the stria terminalis and medial amygdala executes functions related to gonadal steroid actions on the brain; 3) VP originating in the suprachiasmatic nucleus is involved in the regulation of functions that relate to the expression of circadian rhythms. In this paper evidence from recent experiments is presented which supports this concept of place- and origin-coupled function of VP. This conclusion is based on the evidence that VP originating in neurons that are sensitive to gonadal hormones is involved in the regulation of the set point of body temperature. Furthermore, VP originating in the suprachiasmatic nucleus inhibits processes related to activation or stress response.

VASOPRESSIN DISTRIBUTION

Over the past few years many papers have given a description of VP production sites and the distribution of VP terminals in the CNS (see for review Buijs, 1987). Vasopressin synthesis takes place in at least three functionally different areas in the central nervous system: 1) the paraventricular nucleus of the hypothalamus (PVN) where VP is produced in parvo- and magnocellular neurons; 2) the bed nucleus of the stria terminalis (BNST) and the medial amygdala (Ame); 3) the suprachiasmatic nucleus (SCN). For the locus coeruleus (LC) evidence for VP synthesis is rather weak. Until now no evidence has been presented for the availability of VP-mRNA in LC neurons. Since there is no evidence either
that VP is located in terminals of LC neurons, it is very doubtful that this peptide is used as a transmitter by this system. It is therefore not discussed any further in the present paper.

Possible functions of VP originating from the parvocellular PVN in the control of autonomous processes are discussed in the chapter of Dreifuss (this volume; see also Charpak et al., 1989; Hermes et al., 1989b). Here the key position of VP but even more so of oxytocin terminals in the brainstem and spinal cord allows this system to gate afferent and efferent information (see Swanson, 1989; Buijs et al., 1990). For the purpose of the present paper attention is mainly paid to the possible function of VP in the BNST and Ame system on the one hand and the function of VP in the SCN on the other hand.

**VP IN THE BNST AND AME**

The observation of a sex difference in VP innervation in the lateral septum (male rats have a higher density of VP fibres than female ones) was initially attributed to a sex difference in the SCN (De Vries et al., 1981). However, in an attempt to clarify the source of this innervation lesioning of the SCN showed that the origin of VP in the lateral septum was certainly not the SCN. Further lesioning experiments excluded the PVN and subsequently showed that the BNST (where Van Leeuwen and Caffé, 1983, demonstrated the presence of VP neurons, using colchicine treatment) was the source of the VP innervation in the lateral septum (De Vries and Buijs, 1983).

The sexual dimorphic innervation could be attributed to the presence of gonadal steroids early in development. Neonatal gonadectomized animals all developed a female VP innervation pattern that became visible when the animals were substituted later in life with testosterone or estradiol (De Vries et al., 1984). Subsequently it became clear that at any moment in the adult life of a rat the removal of gonadal hormones resulted in the disappearance of the VP content in the systems of the BNST and Ame, whereas the other central VP systems remained unaffected (De Vries et al., 1984). Clearly the functions of VP in these VP systems are linked to the level of gonadal hormones. In search of the physiological role of this system we looked for animals that might change their gonadal hormone levels under natural conditions. This led us to investigate the expression of VP in the BNST and Ame systems of a hibernating animal which shows before the beginning of the hibernation season a dramatic drop in the gonadal hormone level. We thus observed that the drop in gonadal hormone levels seen under these normal physiological conditions can bring down the VP levels in limbic brain regions (Buijs et al., 1986). Since VP is known to be involved in the reduction of fever by infusion of the lateral septum (Naylor et al., 1988; Pittman et al., 1988), we hypothesized that VP is involved in the regulation of the temperature set point. Bilateral infusions were set up whereby animals received small minipump driven infusions in the lateral septum during a four-week period. During this
period no hypothermic bouts could be observed when VP was given, but at the moment when the animals received Ringer infusion or after the VP pumps became empty, hibernation occurred normally (Hermes et al., 1989a). Recently it was demonstrated that VP can also break up a hibernation bout when infused in the middle of such a hypothermic period. Infusion of OT or V2 agonist remained without effect. Infusion of V1A agonist had the same effect as VP, indicating that this effect is truly V1 receptor mediated (Hermes et al., in preparation).

These results indicate that in the lateral septum VP is involved in the regulation of the temperature set point. The absence of VP allows much higher temperatures, as observed in long-term castrated animals (Pitman et al., 1988), or much lower temperatures, as in hibernating animals. Infusion of VP under these conditions reinforces the set point and breaks up the fever or hypothermic period. In these limbic structures VP may be involved in gating hippocampal information to the hypothalamus. Experiments aimed at providing support for this hypothesis failed, however. Infusion experiments with VP in the lateral septum did not result in any change in plasma corticosteroid levels. This result indicates at least that VP is not involved in the regulation of hippocampal PVN-CRF input (Hermes and Buijs, unpublished observation).

The recent observation of Miller et al. (1993) demonstrating the presence of mRNA for galanin in VP neurons in the BNST suggests that this other peptide may also function as a (co)transmitter in this "VP" system. Recently the picture became even more complex because of our finding that the inhibitory transmitter GABA is present in part of the VP terminals in the septal area (Buijs et al., unpublished observation). How this observation can be tied in with the demonstrated excitatory action of VP on septal neurons (Joëls and Urban, 1982; Raggenbass et al., 1987) is unclear at present. It is conceivable, however, that under certain conditions with different stimulation paradigms a neuron may switch from release of a peptidergic transmitter to an amino acid neurotransmitter. In fact such events have been demonstrated for a number of peptidergic amino acid or cholinergic neurotransmitter systems in the peripheral nervous system (Lundberg et al., 1981). We are currently investigating the possibility that GABA synthesis in BNST neurons might also be affected by gonadal hormones, resulting in a different message from these neurons.

**VP IN THE SCN**

It is more than likely that VP originating in the SCN will facilitate or execute functions that may be attributed to this small structure located in the basal part of the hypothalamus just above the optic nerve. Early in the 70s it became clear that the SCN is responsible for the generation of a number of hormonal and behavioural rhythms (Moore and Eichler, 1972) as observed in all mammals kept under a normal light-dark cycle or even under constant
conditions. Recently, data have been presented, from cross-transplantation studies in hamsters with slow and fast free-running circadian rhythms, that provide conclusive evidence that the SCN is the major circadian pacemaker in the CNS. After SCN lesion, making hamsters arrhythmic, the animals received the SCN of their counterparts, which resulted in the ‘slow’ animals becoming ‘fast’ and vice versa (Ralph et al., 1990).

It has been demonstrated that the SCN has an endogenous activity cycle of its neuronal firing rate of approximately 24 h, during which many neurons show a higher activity during the day period and lower activity during the dark period. This periodicity does not depend on the input of the optic nerve and even persists in vitro (Bos and Mirmiran, 1990). In addition, VP release has also been demonstrated to have an endogenous periodicity similar to this rhythm in neuronal activity (Gillette and Reppert, 1987). Therefore, both these circadian rhythms are an endogenous property of SCN neurons.

The SCN consists of a number of different neurons, each type organized in small clusters producing a certain neuropeptide. Thus vasoactive intestinal polypeptide (VIP), somatostatin, gastrin releasing peptide and VP are demonstrated in the SCN (Van den Pol, 1986). The VP projections of the SCN are mainly restricted to the hypothalamus, a major terminal area of VP fibers is present in the rostral PVN and the dorsomedial nucleus of the hypothalamus (DMH) (Hoorneman and Buijs, 1982). Observing the release of VP from

**Fig. 1.** Mean plasma ACTH levels in response to a 15-min infusion of VP pr V1-antagonist in the PVN/DMH area. Infusion of VP in SCN-lesioned animals (left; n=5) causes a clear decrease of ACTH levels as opposed to the infusion of Ringer (Ri). On the other hand, blocking the effect of the endogenously released VP by infusion of a V1-antagonist in intact control animals (right; n=6) causes an immediate rise of plasma ACTH levels.

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SCN neurons during the (subjective) light period we hypothesized that the SCN imposes its rhythm on CNS structures by a rhythmic release of its transmitters in the target structures. In order to test this hypothesis, the SCN was lesioned bilaterally, which removed all SCN-derived input to the hypothalamus. Bilateral cannulas were then placed in the region of the PVN/DMH. Simultaneously, the jugular vein of the rats was cannulated in order to be able to obtain stress-free repeated blood samples. Microinfusions of VP, but not of VIP or Ringer, in the PVN/DMH area resulted in a dramatic decrease of plasma corticosterone, suggesting an inhibiting role of VP in corticosterone secretion (Kalsbeek et al., 1992).

In order to establish the physiological significance of this finding, the same infusion protocol was subsequently applied to intact animals. Here the endogenous VP input was abolished by infusion of a VP antagonist, resulting in a steep increase of the plasma corticosterone level. The involvement of the SCN in this matter was demonstrated by the fact that VP antagonist infusion in SCN-lesioned animals did not result in such an increase in plasma corticosterone (Kalsbeek et al., 1992). Consequently these results indicate that endogenous VP originating in the SCN inhibits corticosterone secretion when released in the PVN-DMH area. Recently, it was demonstrated that this inhibition is largely mediated by the corticotrophin releasing hormone (CRH) containing neurons in the PVN, since ACTH plasma levels show similar changes (Fig. 1) (Kalsbeek and Buijs, 1992). This may fit very well with the observation that VP release from the SCN is the highest during the (subjective) light period even in vitro and with the fact that during this period the lowest blood corticosterone levels are measured. In addition, evidence was provided recently that the SCN, whose electrical activity is also the highest during the light period, has an inhibitory role on basal and stress-induced corticosterone secretion, and that this is more so during the light than during the dark phase of the circadian cycle (Buijs et al., 1993a).

THE ANATOMY OF SCN-PVN INTERACTION

In principle there are several possible (anatomical) routes the SCN may use to transmit the VP signal to the CRH-containing neurons in the PVN. In the first place the most likely candidate is the CRH-containing medial parvocellular part of the PVN itself, which stimulates the release of ACTH after CRH is released into the portal system of the median eminence. However, several anatomical studies have so far failed to provide conclusive data for such a direct SCN-PVN interaction (Watts et al., 1989). In an attempt to investigate the interaction between VP neurons of the SCN and stress-related neurons in the hypothalamus, these ‘stress’ neurons were identified by subjecting animals to a restraint stress followed by Fos immunocytochemistry (Buijs et al., 1993b). This technique revealed all hypothalamic neurons that responded to this stress and enabled us to identify where SCN efferents contacted these Fos-positive neurons. Apart from the parvocellular
neurons in the medial part of the PVN, many neurons exhibited Fos-immunoreactivity, also in the rostral and periventricular part of the PVN, where no CRH-containing neurons are present. In the DMH, too, many neurons exhibited Fos immunoreactivity. Simultaneous Pha-L injection into the SCN or VP immunocytochemistry revealing the SCN efferents showed that Fos-positive neurons in the medial parvocellular PVN received no substantial SCN input. In contrast, Fos-positive neurons in the rostral and periventricular part of the PVN received extensive input from VP SCN neurons. The Fos-positive neurons in the DMH also received VP SCN input as established by VP immunocytochemistry and pha-L tracing.

It can be concluded, therefore, that anatomical data have thus far failed to provide evidence for the existence of a direct connection between the SCN and the CRH neurons in the PVN. However, anatomical tracing studies indicate that the DMH has extensive connections with e.g. these parvocellular neurons in the PVN while the same might be true for the rostral and periventricular PVN (Ter Horst and Luiten, 1986; Levin et al., 1987). We would suggest, therefore, that the influence of the SCN on these neuroendocrine motoneurons is an indirect one, via interneurons located in the PVN itself and in the DMH (Fig. 2). Functionally such an influence via interneurons provides the SCN with more possibilities.

Fig. 2. Proposed pathway used by SCN to induce a diurnal rhythm in ACTH secretion. The dotted lines indicate direct connections between SCN and dorsomedial nucleus of the hypothalamus (DMH) and various parvocellular nuclei of the paraventricular nucleus (PVNp). The periventricular nucleus (Pe) and rostral paraventricular nucleus (rp) are innervated by SCN fibers. The area below the ventral part of the PVN (sp) also receives a direct input. The rp, DMH and Pe have possible or demonstrated projections to the medial parvocellular part of the PVN (mp), where the corticotropin-releasing factor (CRF) neurons are located that release CRF into the portal system of the median eminence. For further details see references and text.
to influence central processes than a direct link towards hormone secreting neurons. These interneurons may relay to many additional structures while otherwise the message is largely "lost" to the periphery. As such, the DMH is an interesting structure, as it has been shown to have reciprocal connections with the ventromedial and lateral hypothalamus, the two antagonistic feeding control centers. The DMH also provides a dense input to circumventricular organs in the forebrain (Luiten et al., 1987). Therefore, it is perfectly positioned to effectuate the message of the SCN to these feeding and drinking centers.

The fact that VP is able to suppress the release of corticosterone in one terminal area of the SCN warrants the question what role this peptide might have in completely different target areas of the SCN, such as the area of the OVLT, diagonal band of Broca and the paraventricular nucleus of the thalamus. We would like to propose that in all these areas VP will more or less support its functions in the DMH, i.e. a function that relates to an inhibition of activity.

It is intriguing that in the process of the control over corticosterone secretion the CNS uses the same neuropeptide in two apparently opposite ways. VP synthesized in SCN neurons and released as a neurotransmitter from its terminals in the PVN/DMH area serves to inhibit corticosterone secretion while the neurons that are (indirectly) reached by this action are also (partly) producing VP, but this time as a neurohormone co-released with CRH to promote ACTH secretion. A solution to this question might be found in studying the evolution of the VP system. It is possible that in other "lower" species VP is also involved in controlling the stress response which has evolved into different control systems during the evolution of the complex circadian pacemaker in mammals.

GABA IN VP TERMINALS

Another interesting aspect of the role of VP as a neurotransmitter in the SCN is the fact that the inhibitory neurotransmitter GABA is colocalized in VP terminals that originate in the SCN (Buijs et al., 1993c). GABA postembedding immunocytochemistry combined with VP preembedding staining enabled us recently to demonstrate at the ultrastructural level that approximately twenty per cent of all VP terminals, originating from the SCN, contained GABA (Fig. 3). Interestingly in a recent study Moore and Speh (1993) demonstrated that all SCN neurons contain the message for GAD, the synthesizing enzyme for GABA. At present the function of this possible co-transmission is unclear. However, the fact that a circadian rhythm exists in the synthesis of VP in SCN neurons together with a circadian rhythm in VP release opens up the possibility that the SCN utilizes VP as a neurotransmitter at one circadian time point and GABA at another. The fact that during the daytime only in a minority of the VP terminals GABA is colocalized indicates that at other circadian time points, the presence of GABA might be more prominent.
Fig. 3. Electron micrograph illustrating a vasopressin positive terminal in the suprachiasmatic nucleus, visible by an electron-dense deposit of DAB, demonstrating GABA immunoreactivity demonstrated by post-embedding 10 nm immunogold deposits (arrows). The VP + GABA terminal contacts an unlabelled dendrite (D).

Obviously, it will be of the utmost interest to investigate whether SCN neurons are able to distinguish between VP and GABA release or whether these neurotransmitters are released in the ratio of their respective concentrations in the terminals.

In conclusion, the present data support the concept that the neurotransmitter functions of VP are closely linked to its site of origin and production and that its function in the target area will heavily depend on the function of that region.
REFERENCES


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