

Vasopressinergic neurons in periependymal and periventricular areas of the rostral third ventricle of the rat

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Summary. On the lateral wall of the rostral third ventricle, an area separates the ependyma from the neurosecretory PVN neurons. Since VP from the latter discharges into the vasculature, the above area may be regarded as constituting an interface between the ventricular and vascular compartments of the CNS. As VP release into the two compartments is integrated, the interface region has been explored for possible existence of a neural infrastructure that would allow such an integration.

Immunohistochemical staining for VP following colchicine treatment reveals the presence of an elaborate vasopressinergic network in the interface region that is divisible into a medial periependymal and a lateral periventricular area. A closer examination indicates that the ependymal, periependymal, periventricular and PVN areas (in that order medio-laterally) are all interconnected through this network. The medial area appears to be receptive in nature, while the connectivity of the lateral area points to an effector function. All in all, such a neural network would provide a sound morphological basis for integration of neuroendocrine mechanisms modulating VP release into the ventricular and vascular compartments of the CNS.

Key words: Vasopressin (VP), Immunohistochemistry, Paraventricular nucleus (PVN), Periependymal area, Periventricular area

Introduction

The paraventricular nucleus (PVN) of the mammalian hypothalamus is concerned with vasopressin (VP) secretion. Modern immunohistochemical techniques using polyclonal or monoclonal antisera to VP have

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greatly aided the identification of vasopressinergic neurons in the magnocellular component of the PVN (Silverman and Zimmerman, 1983; Swanson and Sawchenko, 1983; Morris et al., 1987). However, the region between the third ventricular ependyma and the PVN has received little attention. This region may be considered as comprising a more medial periependymal and a lateral periventricular area. Mention is made in the literature of the presence of a periventricular nucleus in the rostral part of the lateral area (Mitro and Palkovits, 1981). In most immunohistochemical preparations for VP, the ventricular lining, the periependymal and periventricular areas appear largely unreactive. Only a few workers have reported scattered vasopressinergic neurons in these locations (Krisch, 1976; Buijs et al., 1978). Functionally, the latter appears to play an important role in the regulation of body fluid homeostasis, since ablation of the rostral periventricular tissue (AV3V) in the rat leads to complete adipsia and death (Johnson, 1985).

The region is important for an additional reason. Being interposed between the third ventricle and the PVN (whose axons discharge primarily into the neurohypophysial vasculature, and thence into systemic circulation), it constitutes a strategic interface between the CSF and vascular compartments. The source of VP is different for each of these two compartments, although its level in each is affected by identical stimuli (Wang et al., 1982). It is logical to consider that neuroendocrine mechanisms governing VP release in each compartment are integrated and mediated through this region. The present immunohistochemical work investigates the possible existence of a neuronal infrastructure which permits such an integration.

Materials and methods

Antibodies

Primary polyclonal antibody against VP, secondary

antibody (antirabbit Ig raised in sheep) and peroxidase-anti peroxidase (PAP) complex were purchased commercially from UCB Bioproducts, Brussels. The primary antibody was used at a concentration of 1 in 5,000.

Fixation, sectioning and immunostaining

Fifteen adult male Wistar rats (200 g) with free access to food and water were used. Twenty-four hours prior to sacrifice, 10 animals received injections of colchicine (20 µl of 6 mg/ml solution in normal saline-stereotaxic administration) into both lateral ventricles under intraperitoneal pentobarbital (0.4 mg/10 g) anaesthesia. The animals were allowed to recover and were carefully observed for any sign of distress (in which case the animal was killed promptly with an overdose of intraperitoneal pentobarbital and excluded from study). Immediately prior to sacrifice with an overdose of intraperitoneal pentobarbital, all animals were heparinised (0.4 mg/kg) and injected with flaxedil (0.2 ml; 20 mg/ml). Perfusion was carried out through a transcardiac, intra-aortic catheter with approximately 500 ml of a perfusate containing 0.3% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2). During perfusion the animals were kept packed in ice, while artificial ventilation was maintained. Brain blocks, between the optic chiasma and the median eminence, were cut out and were processed sequentially through the perfusate (90 min at 4° C), 4% paraformaldehyde solution (48 hr at 4° C) and 0.1 M phosphate-buffered saline containing 5% sucrose (24 hr at 4° C, pH 7.2).

Seventy-five micron thick vibratome sections were immunostained for VP using the immunoperoxidase technique of Sternberger (1974). Appropriate controls, in which the primary antibody was either omitted or used at very high dilution, (1 in 50,000) were also incorporated. Sections were counterstained with Mayer's haematoxylin and mounted in D.P.X. The procedure has been detailed elsewhere (Ray and Choudhury, 1990).

Results

In rats not treated with colchicine, the ependyma, periependymal and periventricular areas appeared unreactive. The third ventricular ependyma, thus, remained separated from the immunostained PVN neurons by a non-reactive interval (Fig. 1). In occasional untreated rats and in all colchicine-treated preparations, VP-positive neurons were observed in the rostral periventricular area (Fig. 2).

Colchicine treatment revealed marked immunostaining in the otherwise non-reactive interval between the ependyma and the PVN. A large number of VP-positive neurons and their processes were seen to connect these two areas (Fig. 3). On closer observation, immunoreactive neurons were seen lying in the ependyma. Dendritic processes from several of these neurons could be traced towards the ventricular lumen, while their axonal processes pointed in the opposite direction. Isolated

immunostained axons, unrelated to the neurons lying in the ependyma, were also noted as coursing towards the ventricle (Fig. 4). Furthermore, intensely immunoreactive neurons were observed to form a continuous plexus involving the ventricular ependyma and the immediately adjacent periependymal region (Figs. 5, 6).

In the more lateral periventricular region, two features were noted. Firstly, in any given coronal plane through this area, some VP-positive neurons were oriented vertically (dorso-ventrally) and others horizontally (medio-laterally) (Figs. 7, 8). The latter served to interconnect the more medial periependymal neurons with those of the more laterally located PVN. Several axons from the horizontally disposed cells were also noted to be passing on the ependyma towards the ventricular lumen. Secondly, the periventricular area appeared to be traversed by a multitude of VP-positive neuronal processes directly linking the immunoreactive ependymal and periependymal elements with those of the PVN (Fig. 9).

Discussion

It is evident from the foregoing that in rats not treated with colchicine, the amount of immunohistochemically demonstrable VP is low. Consequently, the region between the ependyma and the PVN appears unreactive. Following blockade of axonal transport by colchicine, sufficient neurosecretory material accumulates in this region to permit visualization of neuronal somata and their processes. It becomes apparent that a large number of VP-positive neurons populate this otherwise non-reactive region.

The immunoreactive neurons lying on the ventricular ependyma are noteworthy. These are distinct from tanycytes since the latter are an integral part of the ependyma. Moreover, tanycytes are not known to be vasopressinergic (Krish, 1976). The present finding of dendritic processes extending from the neuronal somata into the third ventricle would suggest that these neurons might play a sensory feedback role in a VP-modulatory mechanism. Because of proximity to the ventricle, such neurons would be well suited to serve as receptors.

The periependymal zone is characterized by an extensive immunoreactive plexus, arranged dorso-ventrally around the rostral third ventricle. The neurons located in the plexus are seen to connect VP-neurons lying medially on the ependyma with those situated in the lateral periventricular zone. Such an arrangement would allow an integration of neural input mediated to the periependymal area either through the presumptive VP-receptors lying on the ependyma or through other neural afferents. It is conceivable that the output is brought to bear on the more laterally located vasopressinergic neurons in the periventricular or PVN areas.

The periventricular zone is of interest for several reasons. The area abounds in immunoreactive neurons that establish contact with the periependymal zone medially and the PVN laterally. As pointed out earlier, the neuronal disposition is characteristic as several of

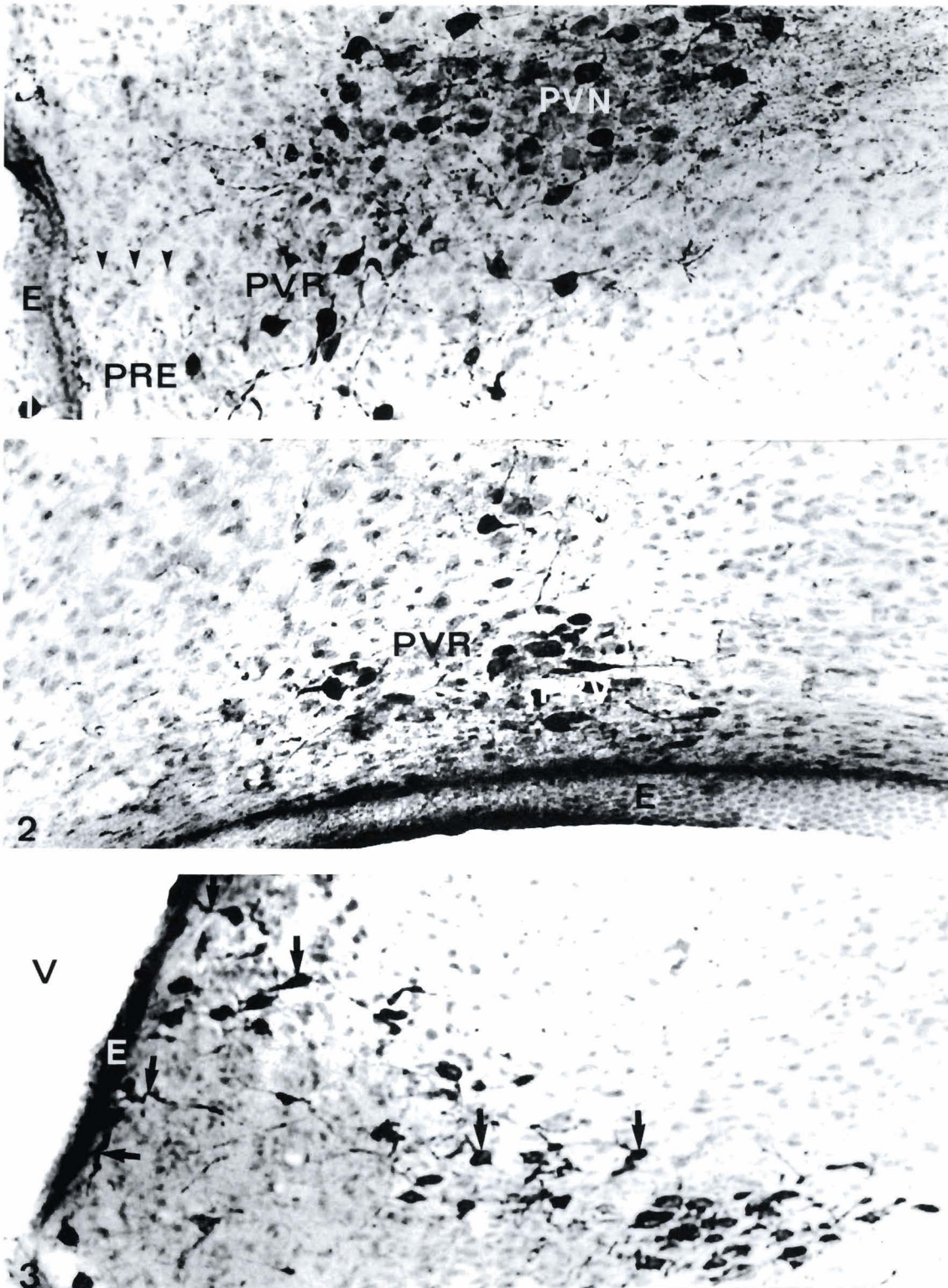


Fig. 1. Lateral wall of third ventricle at the level of PVN from rat not treated with colchicine. Intense immunoreaction for VP is seen at the PVN. Note relatively unreactive region (arrowheads) between the ependyma (E) and the PVN, consisting of a medial periependymal (PRE) and a lateral periventricular (PVR) areas. $\times 354$

Fig. 2. VP immunoreaction in neurons of periventricular nucleus (PRV) in a rat not treated with colchicine. The nucleus is located in the periventricular (PVR) area. The plane of this section is rostral to the one seen in Fig. 1. Ependyma, E. $\times 442$

Fig. 3. VP immunoreactivity in colchicine-treated rat. Note VP positive neuronal soma and processes (arrows) in the periependymal and periventricular areas linking the ependyma (E) and the PVN. Lumen of the third ventricle (V) appears at extreme left. $\times 354$

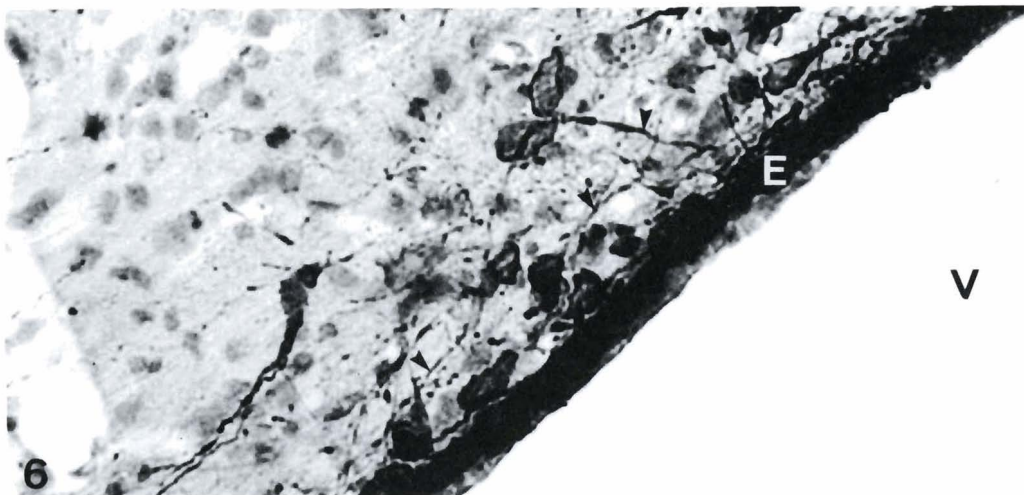
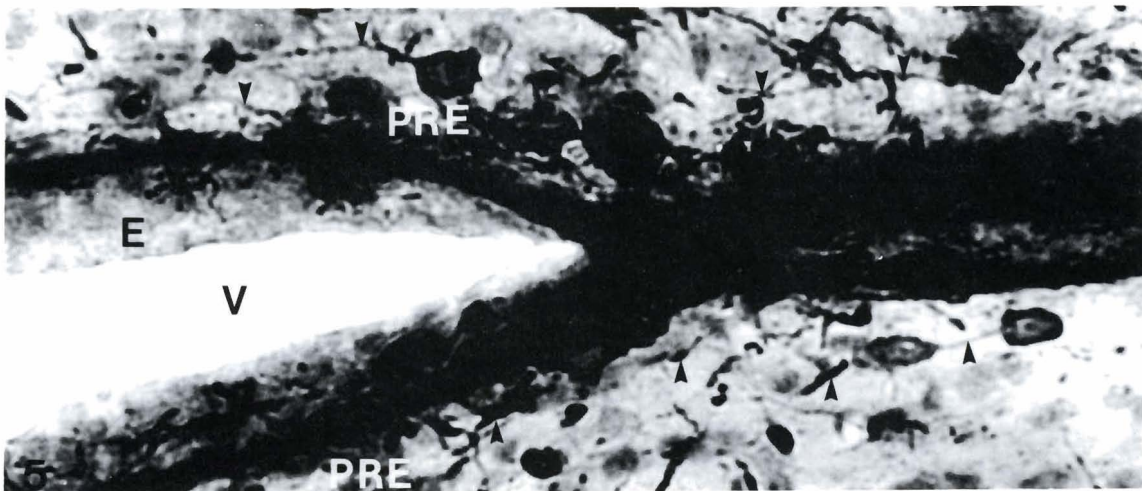
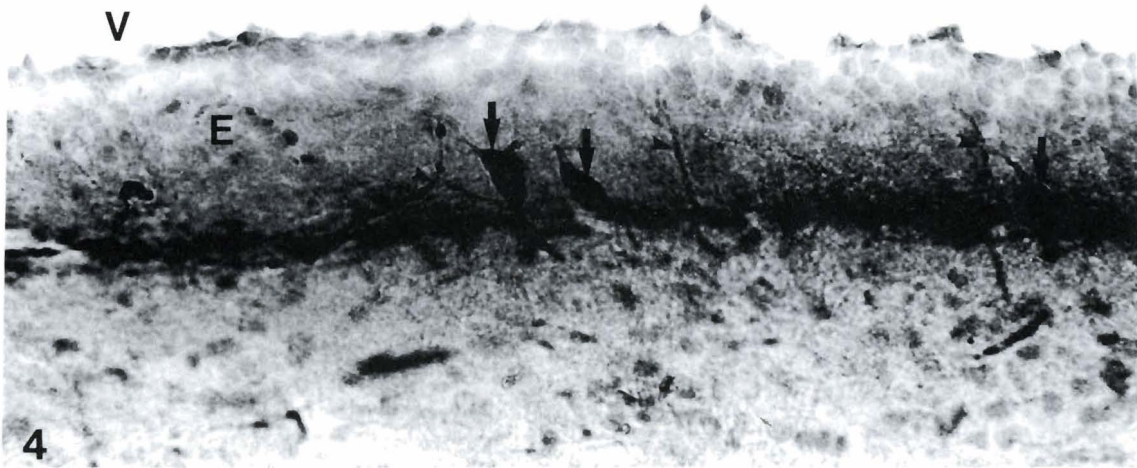
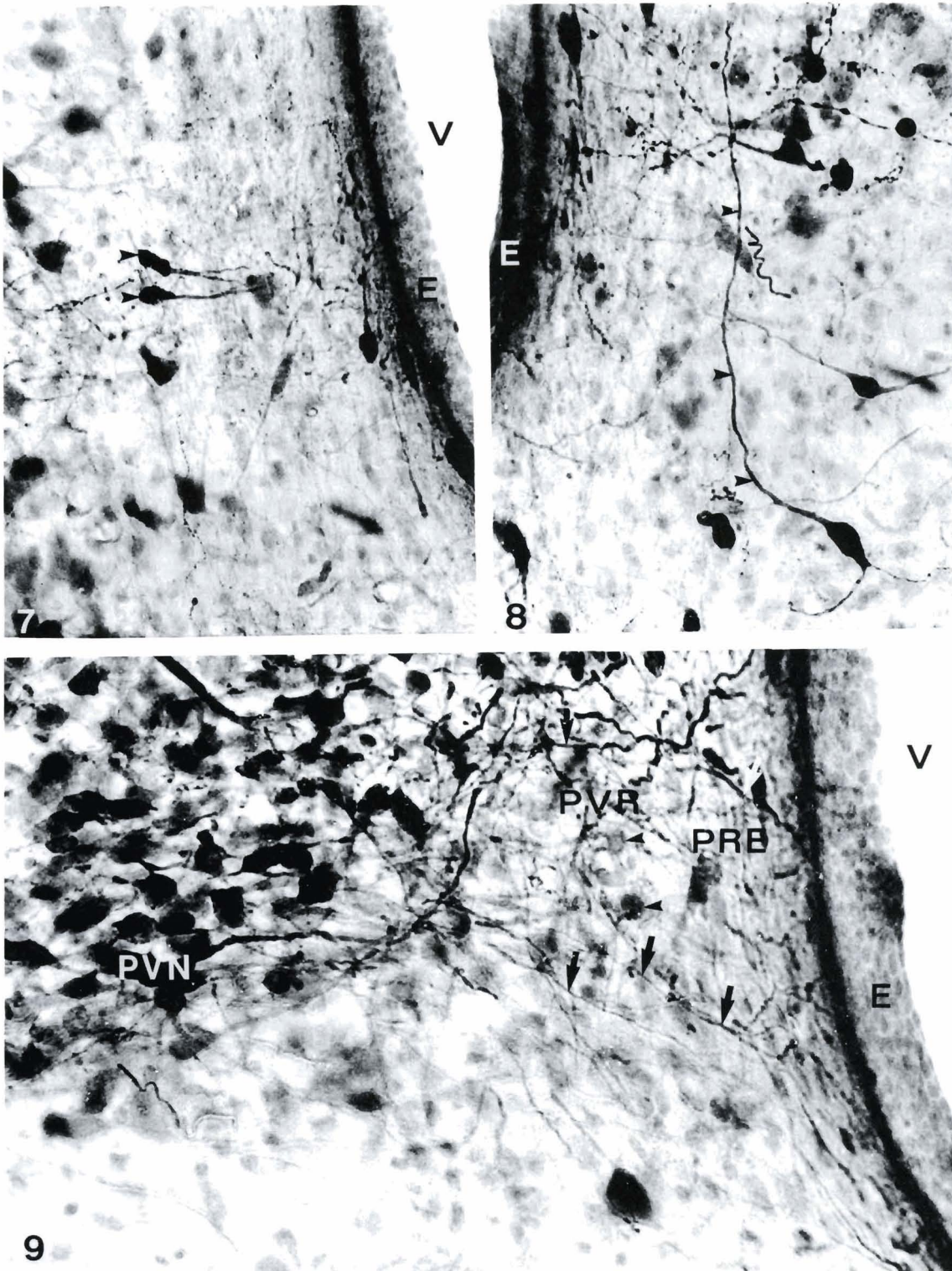


Fig. 4. VP-positive neurons (arrows) lying on ependyma (E) in colchicine-treated rat. Dendritic processes from one neuron can be seen extending towards the ventricular lumen (V). Several immunostained nerve processes (arrowheads) are also seen traversing the ependyma. $\times 885$

Fig. 5. Heavy immunostaining in the ependymal (E) and periependymal (PRE) areas in colchicine-treated rat. The third ventricle (V) and ependyma are disposed horizontally in this picture. The dorsal end of the ventricle is to the left. Note a continuous periependymal plexus (arrowheads) along the dorso-ventral extent of the ventricle. $\times 885$

Fig. 6. Periependymal plexus (arrowheads) in colchicine-treated rat showing marked immunoreaction for VP. Third ventricular lumen (V) is to the right. Note heavy staining in the ependyma (E) due to the superimposition of a large number of immunoreactive neurons. $\times 885$



Figs. 7,8. Periventricular area in colchicine-treated rat. Two horizontally-disposed immunostained neurons (arrowheads) are seen in Fig. 7. A neuron with its axonal process (arrowheads) directed vertically towards the cortex is evident in Fig. 8. Several neurons converge on this vertically disposed axon. Abbreviations as in Fig. 3. $\times 885$

Fig. 9. Lateral wall of third ventricle (V) at the level of PVN in colchicine-treated rat. The section, immunostained for VP, exhibits a multitude of immunoreactive processes traversing the interval between the PVN and the ependyma (E). While some fibres (arrows) interconnect the PVN and the periependymal area (PRE) directly, others do so via the intermediary of neurons (arrowheads) located in the periventricular area (PVR). $\times 885$

their axonal processes course dorso-ventrally or medio-laterally in any given coronal plane. Cells with their axons oriented dorso-ventrally bear a resemblance to the Martinotti cells of the cerebral cortex. Arrangements such as these are conducive to integrative functions within the same area or between adjacent areas. Another point of interest is the observation that a number of axonal processes from the periventricular neurons pass medially to terminate close to the ventricular lumen. It is tempting to speculate that these neurons, among others, may be involved in VP-secretion into the ventricular system. The secretion of isotocin by neurons located in the vicinity of the third ventricle in the pisces is relevant in this regard (Dungen et al., 1982). In mammals, vasopressinergic fibres ending in the choroid plexus, median eminence, lateral and third ventricles, have all been suggested to discharge into the CSF (Wang et al., 1981). The supraoptic, paraventricular and suprachiasmatic nuclei have been mentioned as the possible source for such vasopressinergic fibres (Reppert et al., 1981). However, the source of CSF-VP still remains an enigma (Vokes and Robertson, 1985; Buijs, 1987). Clearly, more work is required in this direction. The third feature of interest in the periventricular area is the passage of a large number of immunostained fibres directly linking the periependymal area with the PVN. It is obvious that the neurons in the latter two areas can communicate with each other directly without the intervention of the periventricular neurons. It would appear that the PVN can be influenced either via a direct periependymal input or indirectly via the intermediary of the periventricular axons.

Taken together, the data demonstrate the existence of an elaborate vasopressinergic network in the ependymal, periependymal and periventricular areas of the rostral third ventricle. The neural connectivity is well geared for integration of modulatory mechanisms operative on the ventricular (CSF) and vascular (PVN) components of VP. In the classical AV3V lesion experiments mentioned earlier, much of the adipsic symptoms are undoubtedly due to the loss of the anterior wall of the third ventricle containing the OVLTA area (Thrasher et al., 1982). However, the adjacent periventricular tissue is also compromised (McKinley et al., 1985). Since this latter area contains elaborate neural circuitry described above, it would be logical to conclude that some of the adverse effects of AV3V lesion also result from the loss of the periventricular area.

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