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Vasopressinergic neurons and the associated blood vessels in the rat anterior hypothalamus: an immunohistochemical study

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Summary. The paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamic neurosecretory system have been extensively investigated by many workers. The functional aspects of vasopressin secretion (elaborated by the PVN and SON neurons) in relation to the vasculature of the anterior hypothalamus are also well documented. However, the available data concerning vasopressin (VP) functions are largely based on physiological studies. Corroborative morphological correlation with regard to this has received little attention. The present report elucidates the intricate anatomical relationships between the VP-neurons and the adjoining capillaries in the rat anterior hypothalamus. A peroxidase-antiperoxidase (PAP) immunocytochemical study, using a commercial VP antibody, was carried out for this purpose. The observations are interpreted from a functional standpoint.

VP-immunostained elements, i.e. the somata and the processes (mainly dendrites), were localized (i) close to the wall, (ii) on the endothelium, and (iii) occasionally, in the lumen of the hypothalamic capillaries. The findings provide immunocytochemical evidence that the vasopressinergic elements are in direct relationship with the hypothalamic vasculature. This raises some interesting possibilities for the former to be involved in: (i) affecting the permeability of the blood-brain barrier for transport of various nutrient substances (important in aging and Alzheimer's disease), (ii) inducing an alteration in the water permeability of the brain vessels on which depends the precise adjustment of brain water content and of brain volume (fundamental to normal functioning of the brain), and (iii) serving as osmoreceptors of the blood flowing through the capillaries and thus providing a feedback mechanism for VP modulation.

Key words: Vasopressin immunocytochemistry, Paraventricular nucleus, Supraoptic nucleus, Capillaries, Rat

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Introduction

Amongst the various nuclei in the central nervous system, the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamic magnocellular neurosecretory system are the most richly vascularised (see Review by Swanson and Sawchenko, 1983). The high density of the capillaries may be associated with the functional requirements in that the magnocellular neurons have a very high metabolic rate, synthesize large quantitaties of hormones and secrete them in the neurohypophysis. One of the hormones, vasopressin (VP), has been reported to modifiy the permeability of the blood-brain-barrier (BBB) for the transport of various nutrient substances (Landgraf et al., 1978; Brust, 1986; Brust and Zicha, 1987; Reith et al., 1987). In addition, VP is known to affect the water permeability of brain microvessels (Rosenberg et al., 1986). All membranes known to regulate water permeability are under the influence of circulating VP (Bindslev et al., 1956). Moreover, it has been recently reported that centrally released VP has profound effects on the water permeability of brain capillaries (Raichle and Grubb Jr., 1978; Dóczi et al., 1982). Furthermore, it is well documented that VP-containing cells in the PVN and SON respond to osmotic stimuli (Brimble and Dyball, 1977; Wakerley et al., 1978). However, these observations are largely dependent on functional studies. Morphological investigation, based on immunohistochemistry, in support of such contentions are sparse. The present work was, therefore, undertaken to elucidate the morphological correlation between the VP-immunoreactive SON and PVN neurons and their surrounding vasculature and, thus, provide an insight into the functional significance of such an association.

Materials and methods

A total of twenty young adult male Wistar rats (200-250 g) were used. The animals were caged individually

and maintained in a controlled environment at 70° F, 45% humidity and with 12 h dark and 12 h light periods. Food and water were given ad libitum. Twenty microlitres of colchicine (6mg/ml) were administered bilaterally into the lateral ventricles of each animal under intraperitoneal Nembutal anaesthesia (0.4mg/10g body weight) 24 hours prior to perfusion of brain tissue. The animals had received heparin (0.4mg/Kg) intraperitoneally 15 minutes prior to intraperitoneal anaesthesia with Nembutal (0.4mg/10g body weight). Under artificial ventilation (90% O_2 and 10% CO_2), the heart and the aorta were exposed. The animals were then perfused with 500ml of a perfusate containing 0.3% glutaraldehyde and 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.2) through a catheter placed trans-ventricularly in the aorta. The animals were kept packed in ice during the entire period of perfusion. Brain blocks, containing the anterior hypothalamus, were dissected out under a stereozoom microscope. These were successively processed at 4° C through the perfusate solution for 90 minutes, followed by 4% paraformaldehyde for 48 hours, and finally 0.1 M phosphate buffered saline (pH 7.2) with 5% sucrose (W/V) for 24 hours. Seventy-five micron thick vibratome sections were cut and immunostained for VP employing the immunoperoxidase technique of Sternberger (1979). Antibody to VP was obtained commercially (U.C.B. Bioproducts, Brussels) and was used at a dilution of 1:5,000. Appropriate controls, in which the primary antiserum was preabsorbed with vasopressin, were also included.

The immunostained sections were counterstained with Mayer's haematoxylin prior to examination under a Leitz Dialux 20 light microscope.

Results

Light microscopical examination revealed intense VP-immunoreaction in the magnocellular neurons of the PVN (Fig. 1) and SON (Fig. 2). Although the immunostaining in the perikarya was observed in the neurons of both the nuclei, the reaction product in the processes was more obvious in the PVN neurons (Figs. 1, 2). In both the nuclei, a striking association between VP-positive neuronal processes and the nearby capillaries was conspicuous. VP-immunostained beaded axons arising from the neurons of the PVN were frequently seen to project towards an adjacent capillary (Fig. 3). While many of these axons skirted around the vessel walls, some ended directly on the endothelium (Figs. 4, 5). In addition, VP-immunoreactive neuronal somata were seen lying in the immediate vicinity of the vessel walls (Figs. 6, 7), while some were located directly on the endothelium (Fig. 8). In some instances, the neuronal cell bodies were found to bathe in the capillary blood with short processes extending into the vascular lumen (Figs. 9, 10). In some cases, VP-positive neuronal processes were seen to traverse long distances in the pericapillary regions and finally to contact the capillary endothelium (Figs. 11, 12). The neuronal cell bodies of such processes, however, were located at some distance from the capillary wall.

Fig. 1. Rat paraventricular nucleus (PVN). Note intense immunostaining for VP in the perikarya and processes of the magnocellular neurons. VP-positive beaded axons (arrowheads) are seen to project mostly in a lateral direction, while many dendritic processes (arrows) are directed towards the third ventricle (3V). × 450

Fig. 2. VP-immunostained rat supraoptic nucleus (SON). Intense staining in noticeable in the magnocellular cells. The reaction product is largely confined to the perikarya (arrows), while only a few processes (arrowheads) exhibit staining. VP-immunoreactivity is also conspicuous in the subpial areas. The optic tract (OT) is seen on the right. × 530

Fig. 3. Rat PVN showing VP-staining in the magnocellular neurons. Lying close by, is a capillary (C). VP-positive beaded axons (arrowheads) arising from the PVN neurons can be seen coursing towards the capillary in bundles. × 475

Fig. 4. A panorama of VP-immunoreactive axons around a capillary (C) in the PVN. While some fibres pass uninterrupted (arrows), others (arrowheads) establish a definite relationship with the capillary wall. A cluster of VP-stained neurons (N) and their processes interconnecting with each other is visible on the upper right. × 425

Fig. 5. An area close to SON showing two capillaries (C) in relationship with VP-positive fibres. The majority of the fibres (arrows) can be seen skirting in bundles around the vessel wall, while a few (arrowheads) are lying on the endothelium. × 520

Fig. 6. A pericapillary region in the PVN exhibiting an isolated VP-immunoreactive neuron (N) with convoluted processes. Two prominent processes (arrowheads) overlie the field of other VP-stained axons traversing this area. The capillary (C) is visible at the top. × 525

Fig. 7. Rat SON. A cluster of neurons (N), intensely stained for VP, is seen lying in the immediate vicinity of a blood vessel (B.V.). The neuronal processes (arrowheads) are visible in the perivascular zone as well as some distance away. × 660

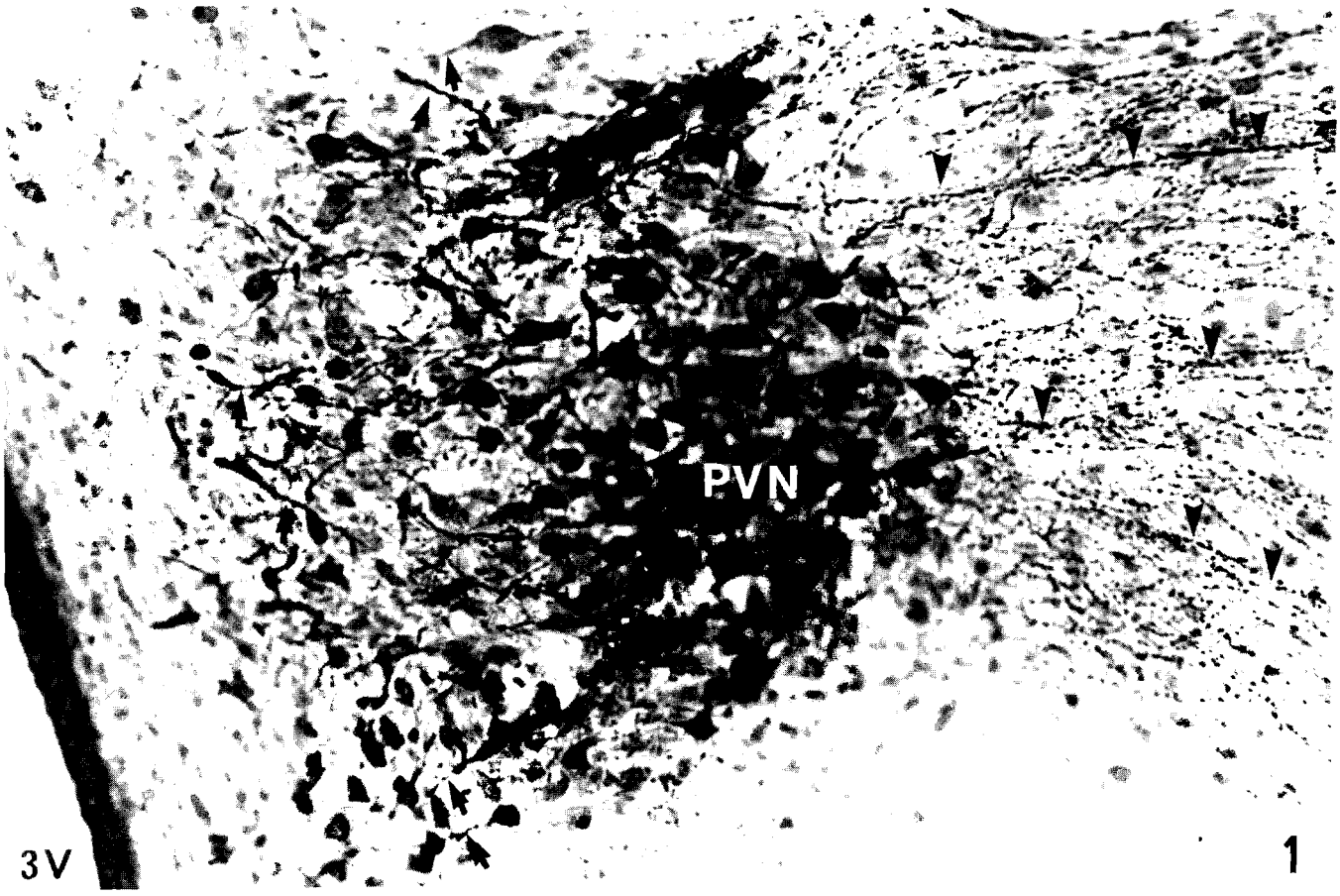
Fig. 8. A group of intensely VP-immunoreactive neurons (N) seen abutting on a capillary (C) in the PVN. A few neurons overlie the endothelium with some processes running parallel to the capillary wall (arrows) and others (arrowheads) towards the lumen. × 580

Fig. 9. Intricate relationship of VP-immunostained neurons with a capillary (C) in the PVN. A cluster of neurons abuts on each bank of the vessel. On the left, two neurons (N) traverse the capillary wall, while two others (N1) lie parallel to the lumen. The cluster on the right (N2) bathes directly in the capillary blood. × 525

Fig. 10. A capillary (C) in the neighbourhood of the PVN in close association with VP-stained neurons. While one neuron (N1) is seen lying across the endothelium, another one (N2) contacts the capillary blood directly. × 525

Fig. 11. Intimate relationship between VP-immunoreactive processes and endothelium of a capillary (C) adjacent to the PVN. A few neuronal cell bodies (N), are seen close to the endothelial wall. Several VP-positive fibres (arrowheads) are seen to travel a long distance before ending on the capillary endothelium. Their parent cell bodies are not visible in this micrograph. × 525

Fig. 12. An area adjacent to PVN. A long beaded VP-stained process (arrowheads) with a right-angled turn on its course is seen terminating on the capillary endothelium. Pericapillary region shows other processes including a recurved one on the lower right (arrows). The somata, from which these processes arise, are not seen in this field. × 470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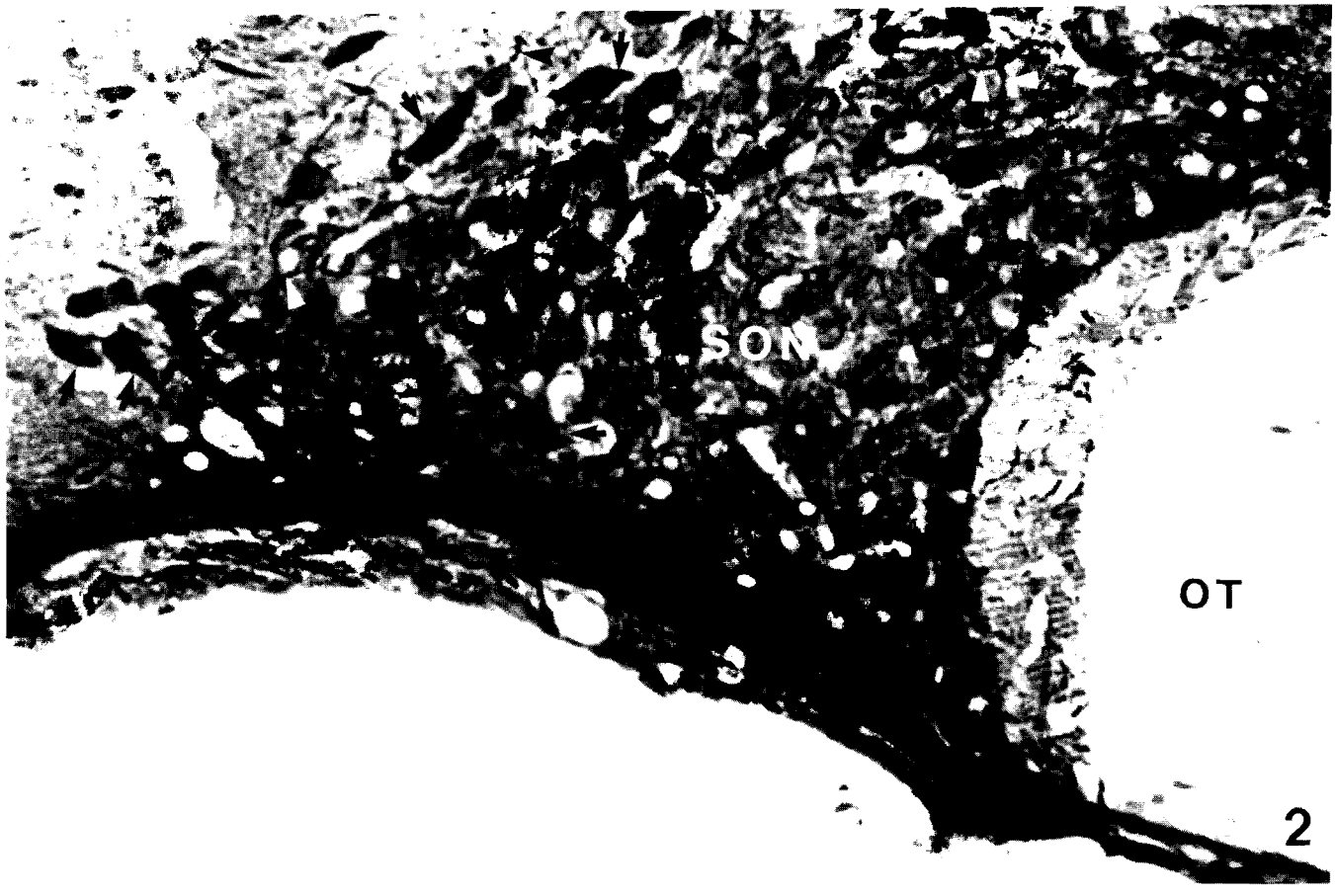
SON
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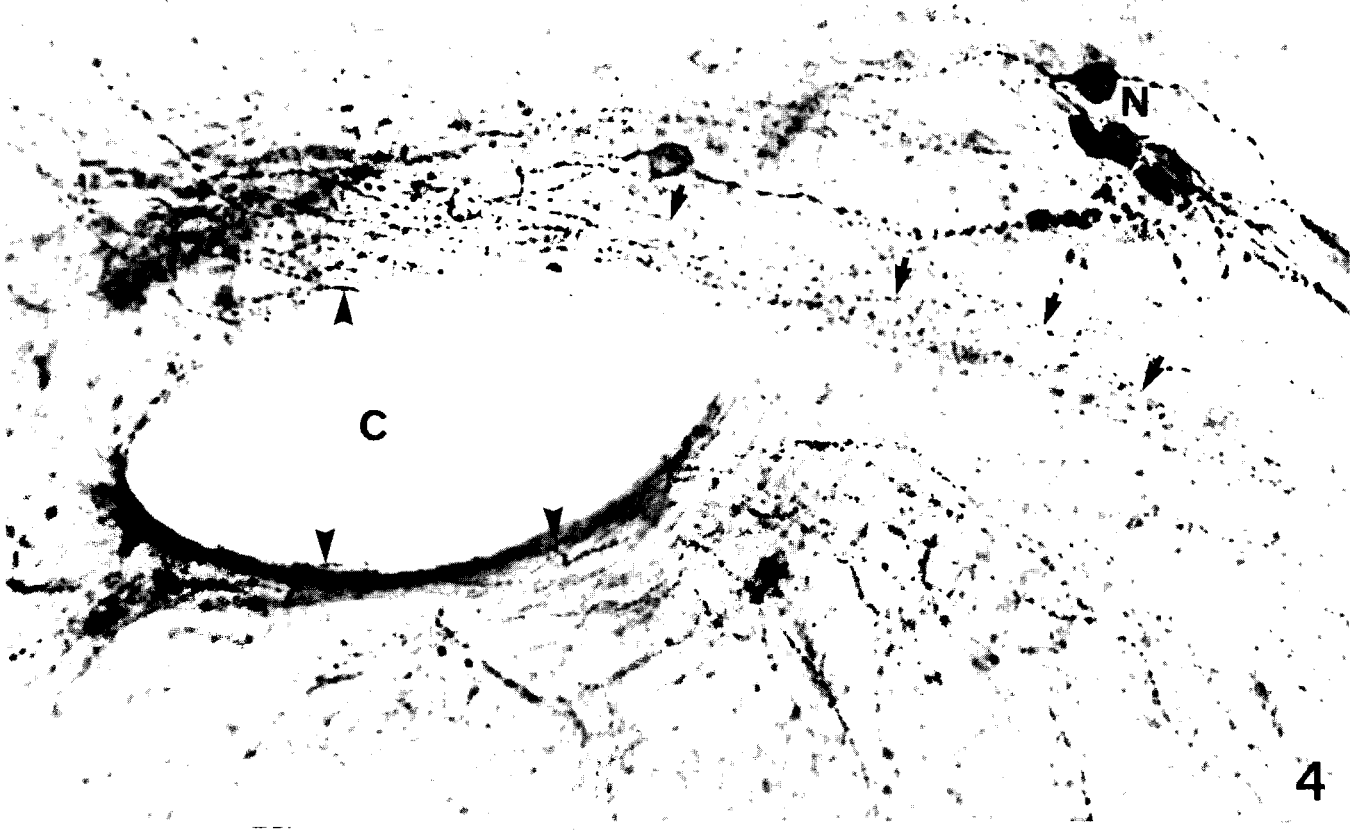

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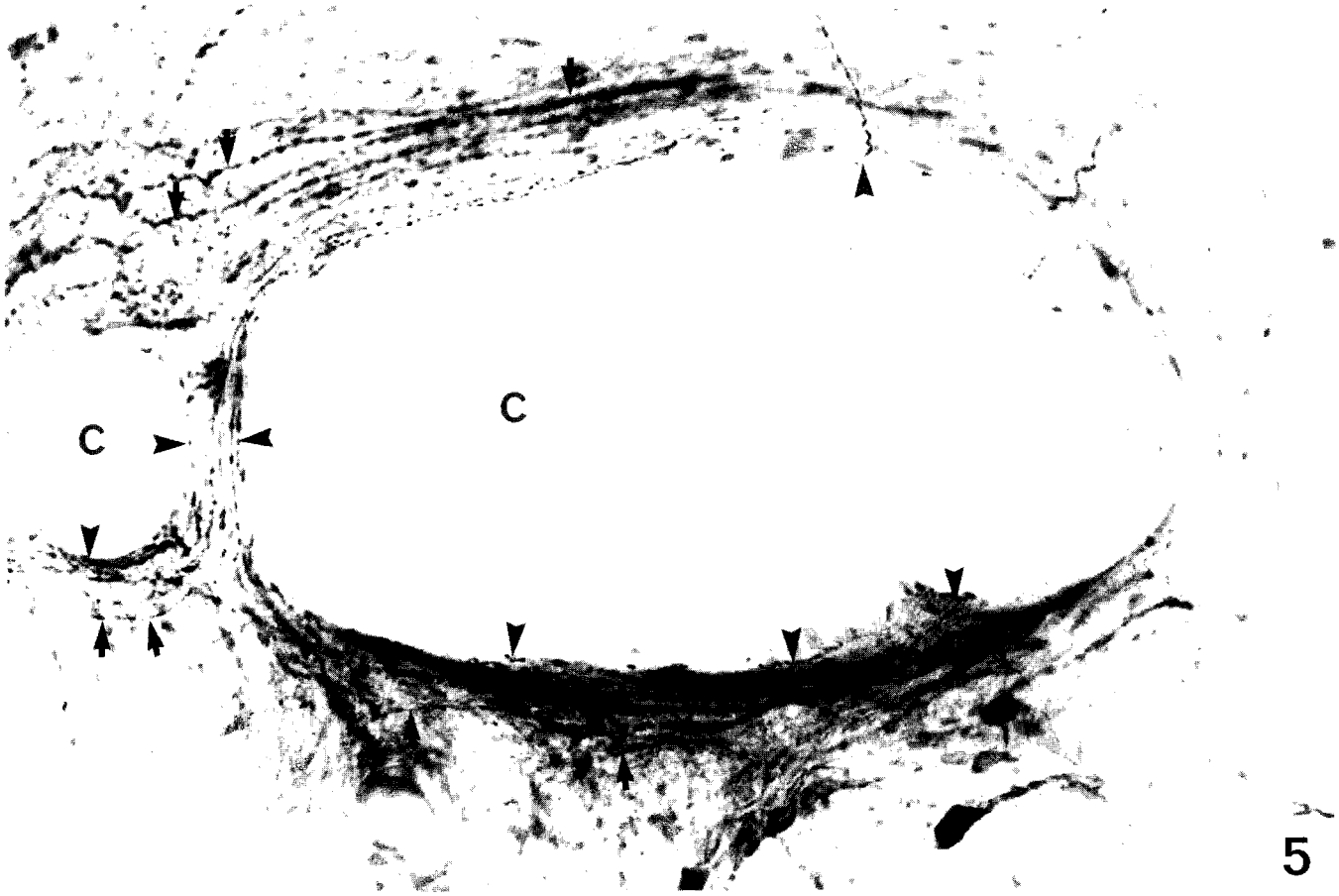

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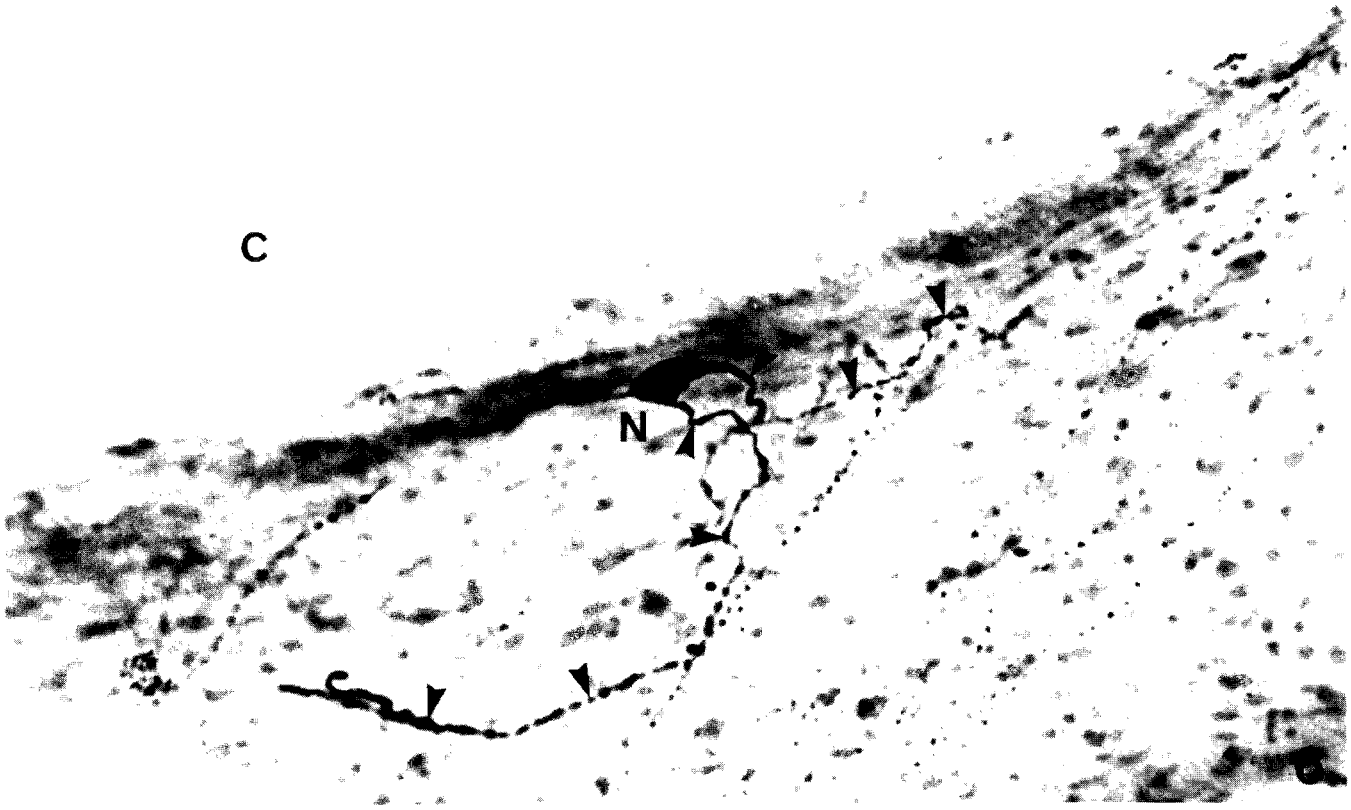
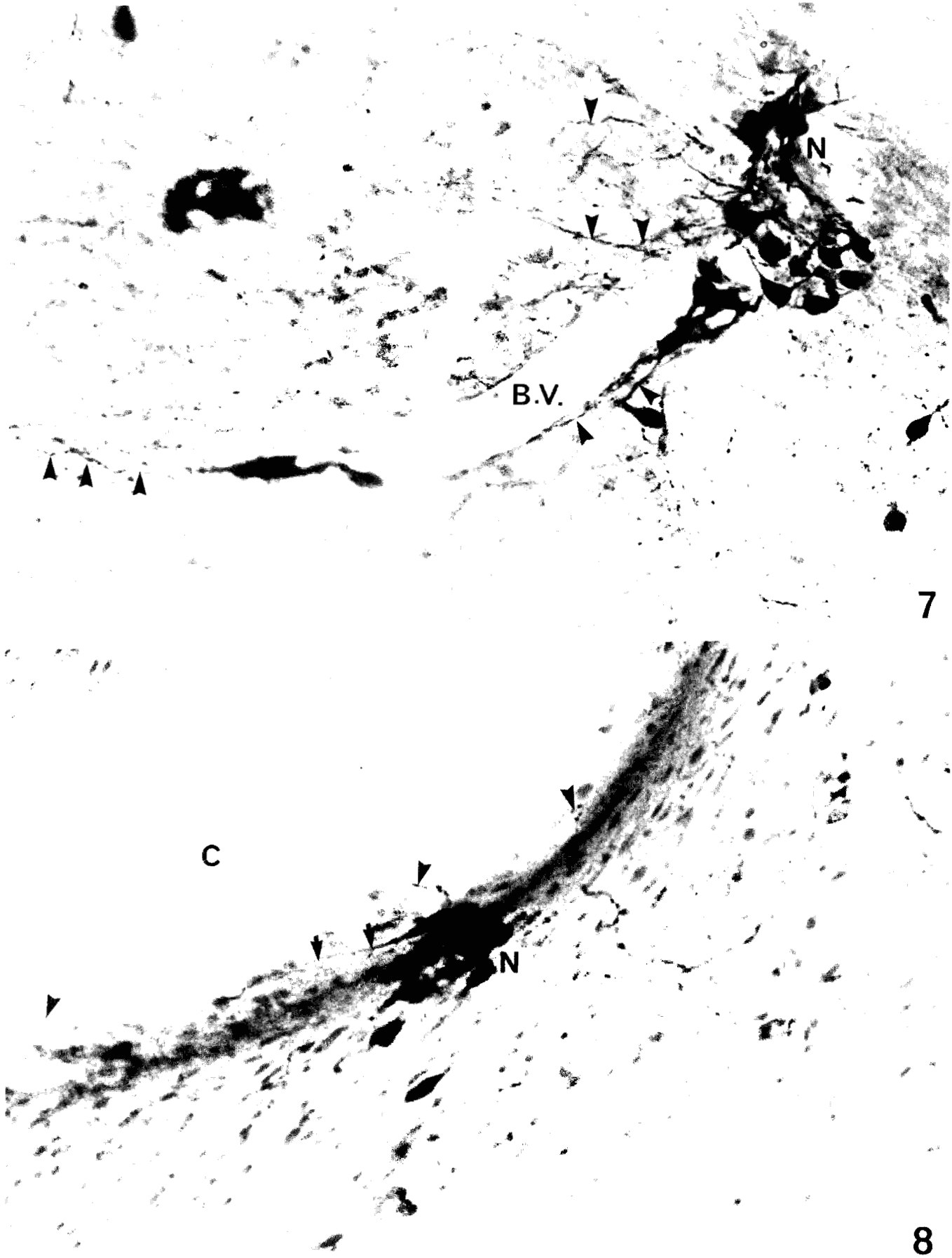
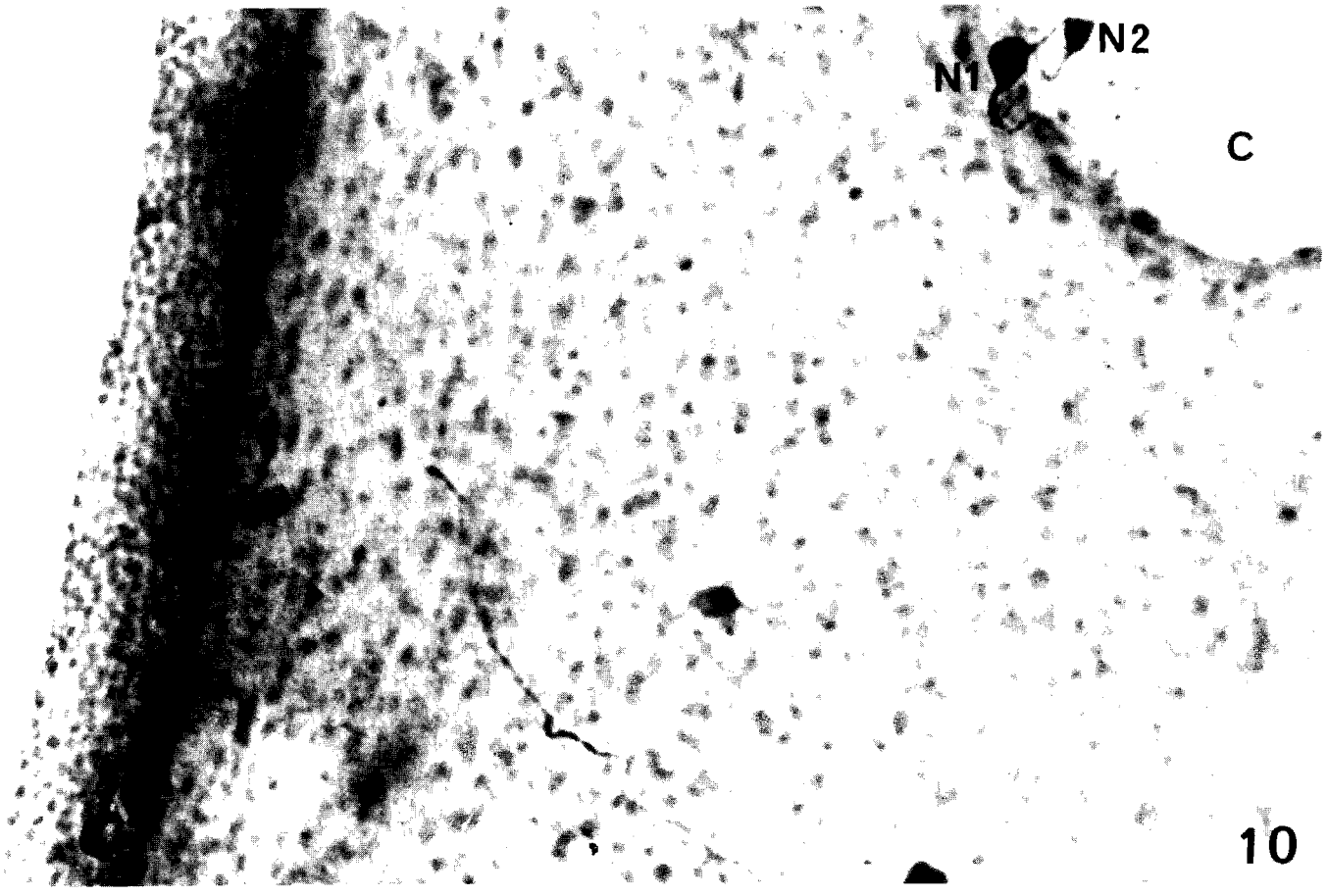
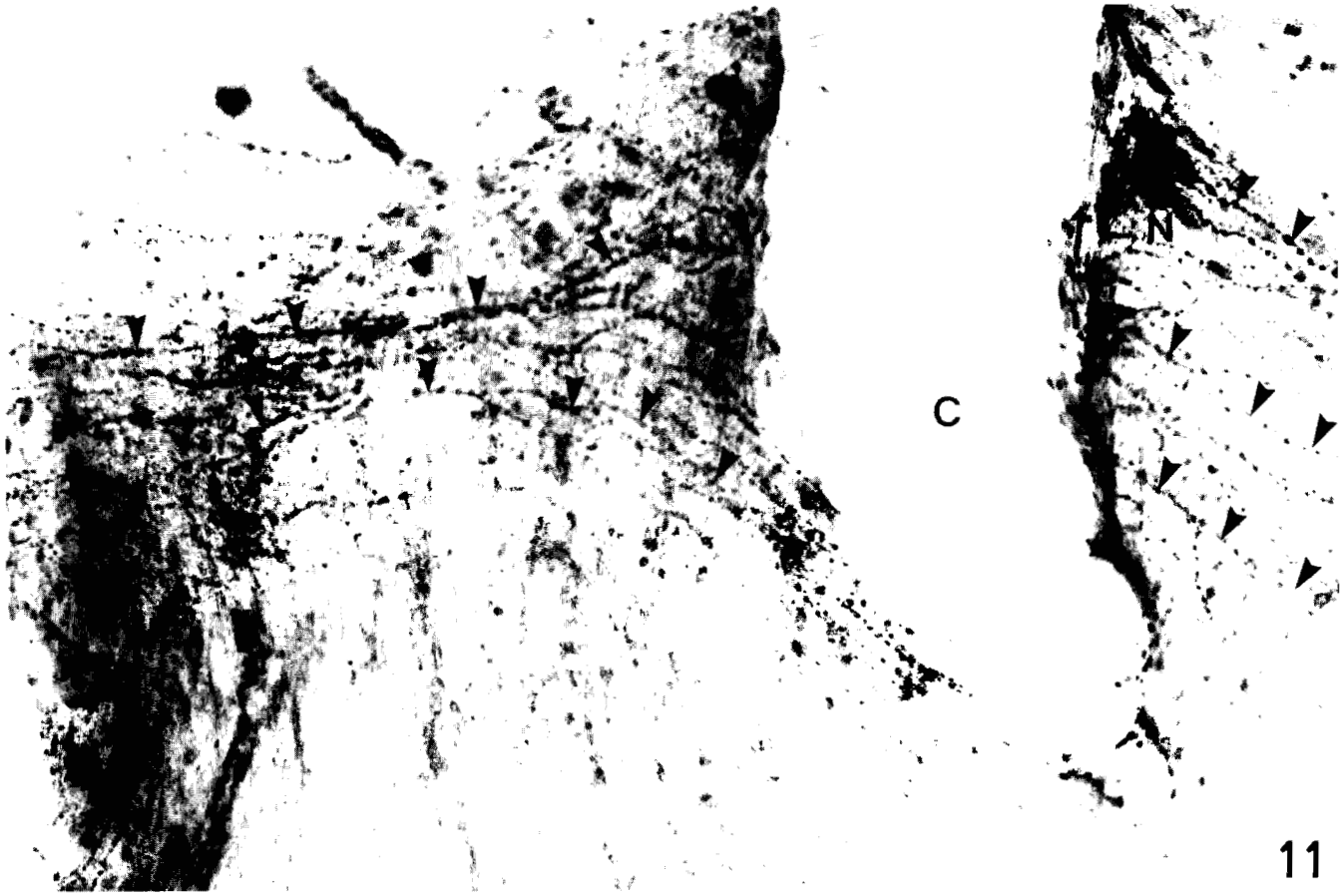



N
B.V.
7
C
N
8


N2
N
N
C
N1
N1
9


N1
N2
C
10



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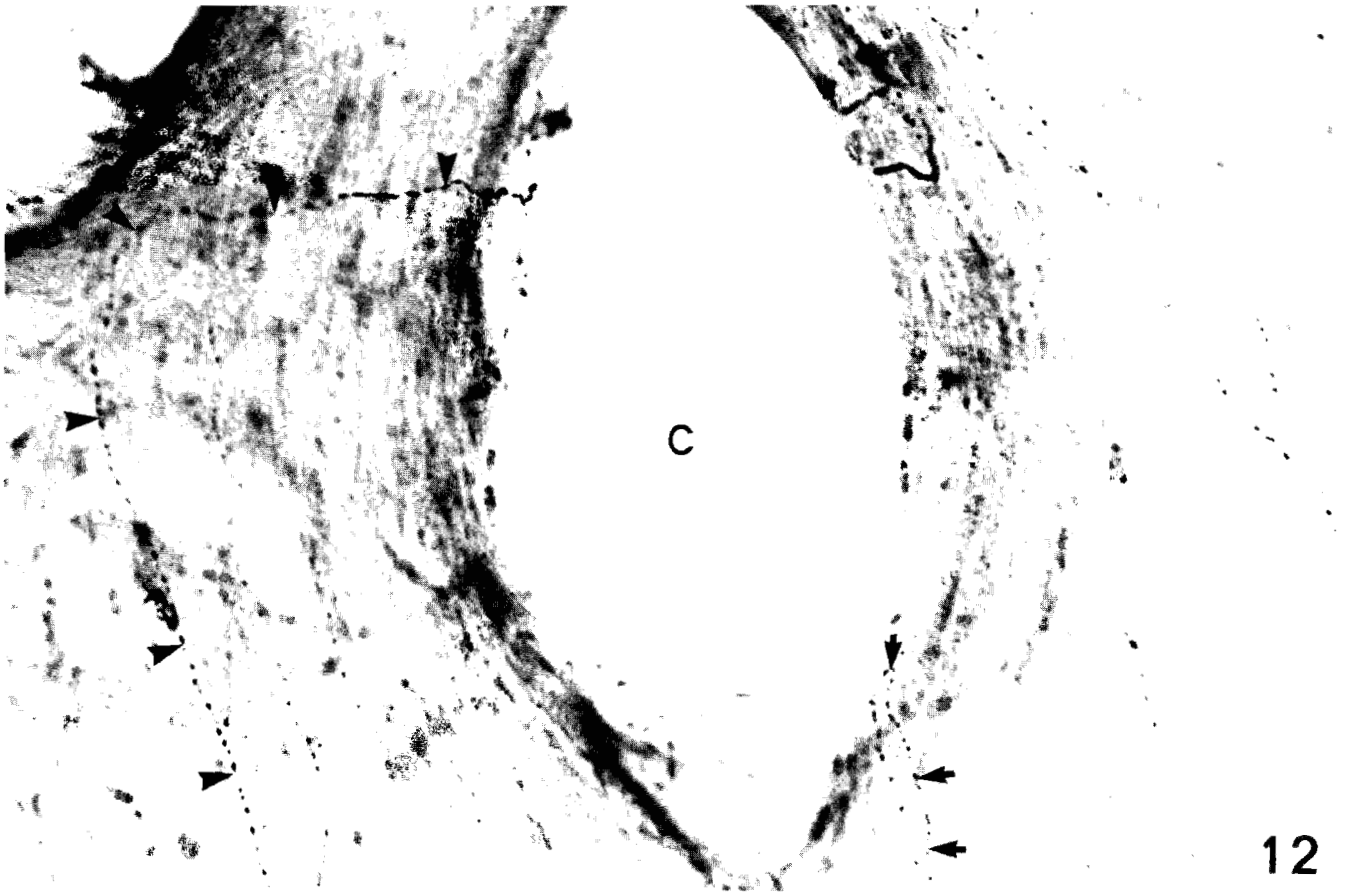

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Discussion

The distribution of VP-immunoreactivity, as recorded in the magnocellular neurons of the PVN and SON, agrees well with the earlier observations of the present authors (Ray and Choudhury, 1987, 1989), and of other workers (Vandesande and Dierick, 1975; Vandesande et al., 1975; Sofroniew and Glasman, 1981; Sofroniew et al., 1981; Swanson and Sawchenko, 1983).

Our demonstration of neuronal somata and processes with VP-immunostaining lying in contact with hypothalamic microvessels is in agreement with the electron and light microscope findings of Krisch (1977), Sofroniew and Glasman (1981) and Jojart et al. (1984). The close association of the VP-immunopositive neurons and of their processes with the microvessels of the anterior hypothalamus is likely to be linked to their role in affecting the permeability of the blood-brain barrier (BBB). Such a mechanism is capable of modifying the transport of various nutrient substances, as documented by Landgraf et al. (1978), Rosenberg et al. (1986), Brust (1987) and Brust and Zicha (1987). Location of VP-stained perikarya and processes on the capillary endothelium, as observed in the present study, parallels with the VP binding sites on the endothelium of blood vessels in the rat brain as seen by van Zwieten et al. (1988). The alterations in the permeability of the BBB are of importance, e.g. in aging and Alzheimer's disease in which functional changes of the BBB have been described (Banks and Kastin, 1986) and in which many changes in VP systems have been reported in both rat and human brain (Fliers et al., 1985a, b).

Besides influencing the BBB, the close proximity of the VP-neurons to the local vasculature is ideally suited to controlling the vascular permeability with respect to water. This is presumably effected through local elaboration of VP. The possibility of central VP release has been postulated by many other workers (Geffen et al., 1976, Krisch, 1980; Suetake et al., 1981; Chapman et al., 1983). The VP, released locally, would appear to bring about changes in the capillary endothelium by indirectly acting on their noradrenergic innervation (Miller et al., 1979; Church, 1983).

The observed closeness of VP-positive elements and the capillaries in the anterior hypothalamus is a pointer to another interesting possibility: the neuronal perikarya and dendritic processes may be sensitive to osmotic stimuli. This hypothesis is supported by the electrophysiological data of Brimble and Dyball (1977) and Wakerley et al. (1978) which confirmed that VP-containing cells in the PVN and SON respond to osmotic stimuli. More recently, it has been established that SON neurons are themselves directly osmosensitive and part of an osmoreceptive complex (Mason, 1980). The localization of VP-immunostained cell bodies and processes abutting on the hypothalamic capillaries suggests that diffusion of substances out of the capillaries or the osmolarity of the blood flowing through the capillaries may affect these neurons either by stimulation or inhibition.

The pericapillary VP-neurons may secrete VP directly into the lumen of the vessels as suggested by Muchlinski et al. (1980). The released VP may then be transported to the PVN and SON through the blood stream, and may modulate the function of VP neurons in those nuclei.

Lastly, the VP-positive fibres which travel long distances to end in the capillaries merit further consideration. These are strategically located to monitor the VP concentration in the plasma, and accordingly influence VP synthesis by the SON and PVN neurons. Thus, these may be regarded as constituting an afferent arc in the feedback mechanism for VP regulation.

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