

Review

Perivascular nerves and vascular endothelium: recent advances

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Summary. Within the last few years, advances have been made regarding perivascular nerves and the endothelium of the vascular system, both potentially important in the understanding of the mechanisms of local control of blood flow. Endothelin-1 (ET-1) has been identified in rat cerebrovascular nerves, neuropeptide Y (NPY) has been demonstrated in umbilical endothelium, the arginine-vasopressin (VP) system has been discovered in the heart (including coronary endothelium), and P2X receptors have been observed in vascular endothelial cells. After a brief introduction to vascular biology, this review will focus on the above-mentioned new data.

Key words: Perivascular nerves, Endothelium, P2X Receptors

Introduction

Since the early 1980s, major advances in vascular biology have been made. In 1980, Furchgott and Zawadzki found that acetylcholine (ACh) stimulates aortic endothelium to release an endothelium-derived relaxing factor (EDRF) that mediates vasorelaxation. The identity of EDRF was later determined to be nitric oxide (NO), produced by an L-arginine pathway that uses the enzyme NO synthase (Palmer et al., 1987). In 1988, Yanagisawa and colleagues discovered endothelin-1 (ET-1), a potent vasoconstrictor of endothelial origin which together with vasorelaxant NO, appear to be essential for the maintenance of cardiovascular homeostasis (see Rubino et al., 1999). In fact, the cardiovascular system is controlled by a number of vasoactive agents released from both endothelial cells (e.g. an endothelium-derived hyperpolarising factor (EDHF), prostacyclin, endoperoxidases, superoxide anions and thromboxane A₂), and sympathetic,

parasympathetic, sensorimotor or intramural perivascular nerves (see Burnstock, 1990a,b, 1999; Vanhoutte, 2000). Consequently, the terms 'neural-endothelial interaction' and the 'dual control' of blood flow now have a permanent place in vascular biology (see Ralevic and Burnstock, 1993; Burnstock and Ralevic, 1996).

Perivascular nerves: ET-1

In 1998, a new 'type' of perivascular nerve, containing ET-1, was discovered (Loesch et al., 1998). By means of electron-immunocytochemistry, it was shown that ET-1 is present in a subpopulation of perivascular nerves of rat basilar artery (Fig. 1). Studies of the basilar artery of spontaneously hypertensive rats have revealed an increase in ET-1-positive perivascular axons, although these axons usually showed abnormalities (Milner et al., 1999). It is not yet known however, whether an increased neural source of ET-1 in cerebral arteries of hypertensive animals contributes to development of hypertension or whether it is a consequence of selective degenerative changes (Milner et al., 1999). It will be important to establish the role of ET-1 of neural origin in these mechanisms. There is evidence to suggest that ET-1-containing cerebrovascular nerves originate from a sensory component of trigeminal ganglia-primary afferent sensory neurones (Milner et al., 2000). It should be noted that cerebral arteries receive rich innervation consisting of sensory, sympathetic and parasympathetic neurotransmitters/neuromodulators, including vasodilator nerves that produce NO (Burnstock, 1990c). A variety of vasoactive agents, as well as NO synthase, have also been identified in cerebrovascular endothelium, making cerebral vessels well-equipped with sensitive mechanisms to control local blood flow (Burnstock, 1990c; Loesch and Burnstock, 1996, 1998a). Perivascular nerves of cerebral arteries should now be recognised as a source of cerebrovascular ET-1, in addition to the endothelial source of this neuropeptide (Loesch et al., 1998; Milner et al., 1999). Recent studies of ET-1 innervation to cerebral arteries suggest that this type of innervation is also present in human (*post-mortem*) cerebral arteries

(Loesch and Burnstock, 2002).

Vascular endothelium

In 1985, Parnavelas and colleagues demonstrated for the first time, the presence of immunoreactive choline acetyltransferase (ChAT - the enzyme synthesising ACh) in endothelial cells of small cerebral vessels of the rat, suggesting that the endothelium can be a source of circulating ACh (Parnavelas et al., 1985). Before this, it had been assumed that circulating ACh is derived primarily from perivascular nerves. However, it later became unclear whether ACh and other vasoactive agents could travel freely from perivascular nerves to the circulation and/or intima, to stimulate release of EDRF (see Burnstock, 1987a,b; Lincoln and Burnstock, 1990). For example, muscarinic receptors on smooth muscle of the media can intercept ACh of perivascular origin causing vasoconstriction. It seemed, relevant therefore, to investigate whether the source of the agents implicated in the endothelium-dependent responses was localised elsewhere; namely, whether the endothelium itself contains these agents and whether it releases them. According to Saetrum Opgaard and colleagues (1998), the endothelium is a likely source of a variety of vasoactive agents. They also suggest that the amount of vasoactive agents measured in general or local circulation may not reflect the amounts released by perivascular nerves (Saetrum et al., 1998).

Immunocytochemical studies of various vascular beds have detected several classical neuropeptides, non-

peptide substances and/or enzymes of vasoactive agents in intact and/or cultured endothelial cells (see Loesch and Burnstock, 1998a). These include VP (a neuropeptide commonly identified as an antidiuretic and vasoconstrictor hormone, produced and released into the circulation within the hypothalamo-neurohypophysial system), NPY (a sympathetic co-transmitter), calcitonin gene-related peptide (CGRP) and substance P (SP) (both sensorimotor neurotransmitters and/or neuromodulators), vasoactive intestinal polypeptide (VIP, a parasympathetic cotransmitter with ACh) as well as atrial natriuretic peptide (ANP), angiotensin II (Ag II), 5-hydroxytryptamine (5-HT), histamine, ET-1, ChAT and NO synthase I and II. It has clearly been shown that the substances localised in the vascular endothelium, such as SP, 5-HT, ET-1, VP and ACh, can be released from intact and/or cultured endothelial cells under the conditions of changing vascular tone (see Bodin et al., 1994; Milner et al., 1997). For example, early studies of the venous effluent of the Langendorff heart preparation demonstrated substantial release of ACh, SP and 5-HT, as well as ATP during hypoxia (suggesting an endothelial origin of the substances) whilst immunoreactivity to SP, ACh and 5-HT have been localised in coronary endothelium (Burnstock et al., 1988; Milner et al., 1989).

Endothelial NPY

Six years after the discovery of immunoreactive NPY in endothelial cells of the rabbit central ear artery

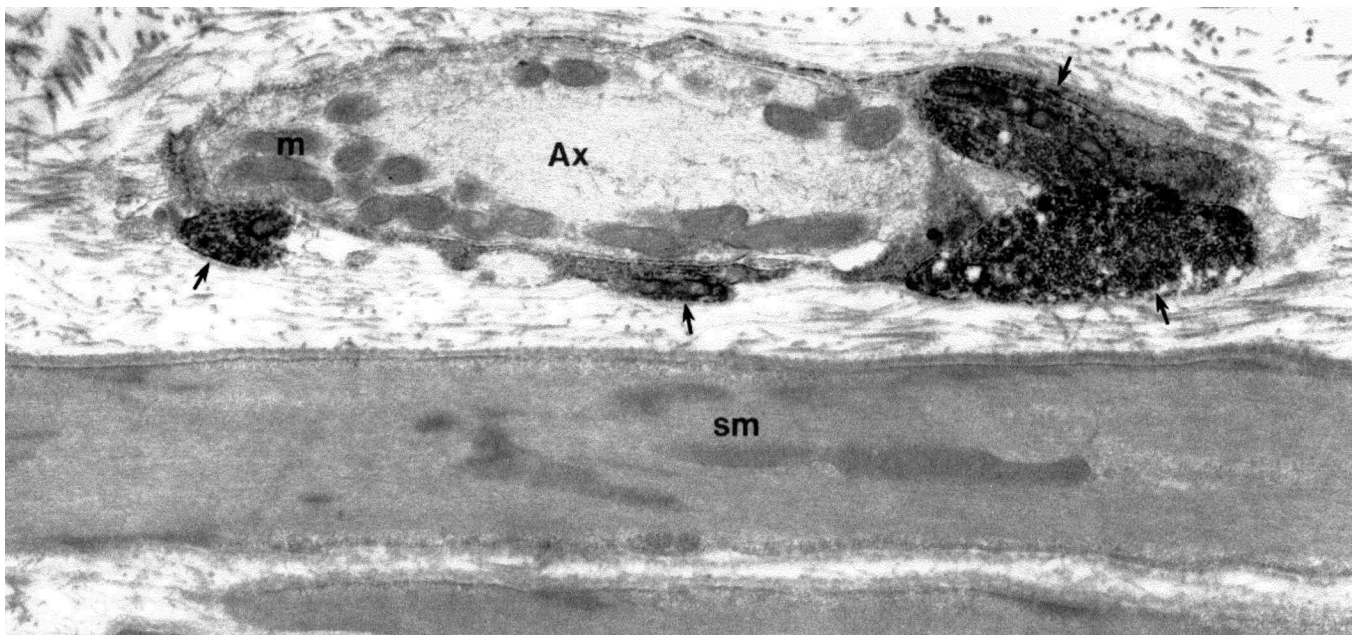


Fig. 1. Electron-immunocytochemical localisation of ET-1 (PAP method) in perivascular nerves of rat basilar artery. Note at least four ET-1-positive (arrows) and at least one ET-1-negative (Ax) axon profiles in the nerve bundle close to the smooth muscle (sm). m: mitochondria. (The role of ET-1-positive nerves has not yet been determined – e.g. vasoconstrictors?). For more information about ET-1-innervation of basilar artery see: Loesch et al., 1998). x 15,000

(Fig. 2) following chronic electrical stimulation of great auricular nerve supplying the artery (Loesch et al., 1992), the presence of NPY mRNA and immunoreactive NPY was demonstrated in cultured human umbilical vein endothelial cells (Zukowska-Grojec et al., 1998). According to Zukowska-Grojec and colleagues, NPY possesses strong angiogenic properties, suggesting a role for this neuropeptide in angiogenesis during tissue development and repair. This is an important discovery of a new function for NPY in the cardiovascular system, particularly with respect to the umbilical vein. The umbilical vein and central part of the maternal end of the umbilical cord are not innervated; only the fetal side of the umbilical cord receives NPY-, CGRP- and tyrosine hydroxylase-positive fibres (Sato, 1998). Thus, the source of NPY in the umbilical vein (Lin et al., 1991; Kokot et al., 1998) should not be linked solely with perivascular nerves.

Studies by Cai et al., (1993) showed that about 32%

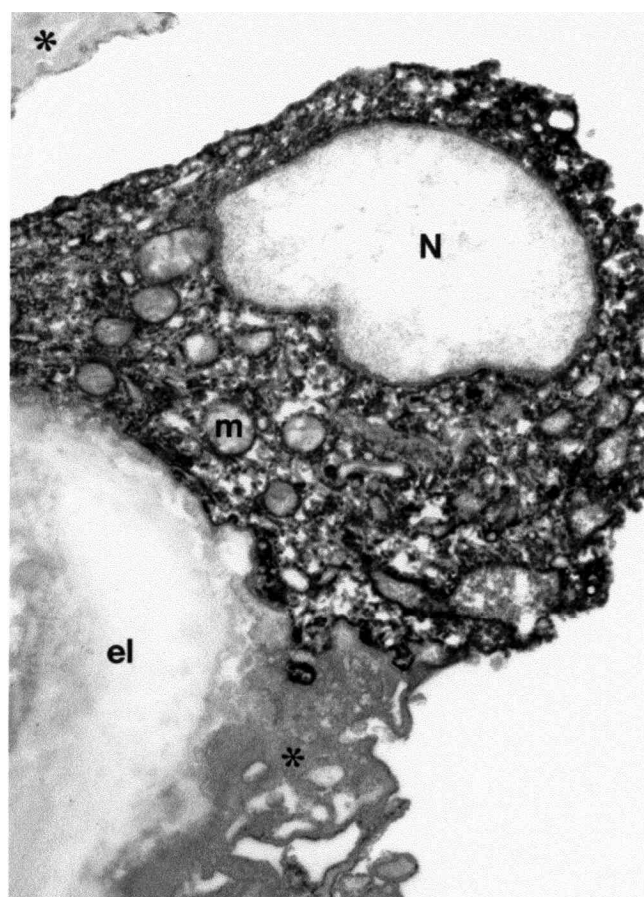


Fig. 2. Electron-immunocytochemical localisation of NPY (PAP method) in endothelial cells of rabbit central ear artery following long-term (16 days) electrical stimulation of perivascular nerves in vivo. A fragment of artery shows one NPY-positive (black cytoplasmic stain) and two NPY-negative (asterisks) endothelial cells. N: nucleus; m: mitochondria; el: elastic lamina. For more information about NPY-immunoreactivity in rabbit central ear artery see: Loesch et al., 1992. x 19,000

of intact endothelial cells of human umbilical vein from term pregnancies are NPY-immunoreactive. It is not known whether the umbilical endothelium releases NPY. However, if NPY were released from umbilical vein endothelium, speculation about the influence of NPY on the vascularisation of the fetus is relevant. Further studies of NPY in early gestation would contribute to our understanding of factors influencing fetal development. In fact, it seems likely that umbilical endothelium (including that of the umbilical vein) provides a variety of vasoactive agents to the umbilical circulation, which in turn may influence the vascular tone and hence blood flow to the fetus (see Loesch and Burnstock, 1996).

Endothelial VP

In the late 1980s, the first immunocytochemical studies of VP in intact endothelium were performed, resulting in the demonstration of VP localisation in rat renal, mesenteric and pulmonary arteries (Fig. 3) (Lincoln et al., 1990; Loesch et al., 1991). Recently Hupf and colleagues (1999) from Germany provided further support of a claim that VP can be produced by intact endothelium. They identified VP protein and mRNA in the rat heart and reported on VP localisation in coronary endothelium. Furthermore, they provided the evidence for the *de novo* synthesis and release of VP into the coronary circulation of isolated hearts, in particular when acute pressure overload or NO stresses are applied to the heart. According to Hupf et al. (1999), VP of cardiac origin may 'counterbalance' the action of NO in the heart by being implicated in coronary vasoconstriction and impaired relaxation. Because an NO synthase inhibitor (L-NAME) can block VP synthesis in the heart resulting in reduced VP levels in pressure overloaded or NO stressed heart (Hupf et al., 1999), there may be important clinical consequences, e.g. the methods employed to deliver arginine therapy in cardiovascular disease. Investigation of the role of 'cardiac' VP in coronary vessels is therefore justified. For example, examination of the fine-ultrastructural distribution of immunoreactivity to NOS and VP may help in assessing the extent of the relationship between cardiac NO and VP in pathophysiological circumstances. In the older population, for example, heart failure is the most prevalent cardiovascular disorder (Rossi, 2000). Both in animals (rat) and humans, cardiac failure is accompanied by increased plasma VP and hypothalamic VP mRNA (Schrier et al., 1998). It is possible that VP of cardiac origin (Hupf et al., 1999) also contributes to elevated VP in plasma and heart failure in ageing.

Discovery of the VP system (VP synthesis and release) in the heart raises the possibility that VP is produced by the coronary endothelium with potentially systemic effects (Hupf et al., 1999), in addition to the effects produced by VP release from the hypothalamo-neurohypophysial system. Electron microscopy studies of newborn rat heart clearly show localisation of VP in endothelial cells of main coronary arteries, cardiac

microvessels and in fibroblast/fibroblast-like cells close to coronary vessels (Loesch and Burnstock, 1999, 2000a).

Endothelial P2X receptors

It is well established that extracellular purines and pyrimidines are involved in intercellular signalling, mediating the control of vascular tone (Burnstock, 1990b, 1997; Abbracchio and Burnstock, 1998; Ralevic and Burnstock, 1998). A purine adenosine 5'-triphosphate (ATP) is probably the most prolific vasoactive agent synthesised in endothelial cells (Paddle and Burnstock, 1974; Pearson and Gordon, 1985). ATP is also actively released from endothelial cells, in

particular in response to shear stress (Bodin et al., 1991, 1992; Bodin and Burnstock, 1995). It is known that ATP stimulates two families of receptors, the cation-selective channels, called P2X receptors (seven subtypes recognised) and the G-protein-coupled P2Y receptors (six subtypes recognised) (Abbracchio and Burnstock, 1994; Burnstock and King, 1996). P2Y receptors are well known to mediate NO production and hence, to induce vasorelaxation (Malmjö et al., 1999). The vasodilatory action of extracellular ATP is claimed to be mediated via P2Y receptors on endothelial cells, whilst vasoconstriction is mediated via P2X receptors on vascular smooth muscle (Burnstock and Kennedy, 1986). Endothelium generally expresses P2Y₁, P2Y₂ and P2Y₄ receptor subtypes (see Ralevic and Burnstock, 1998).

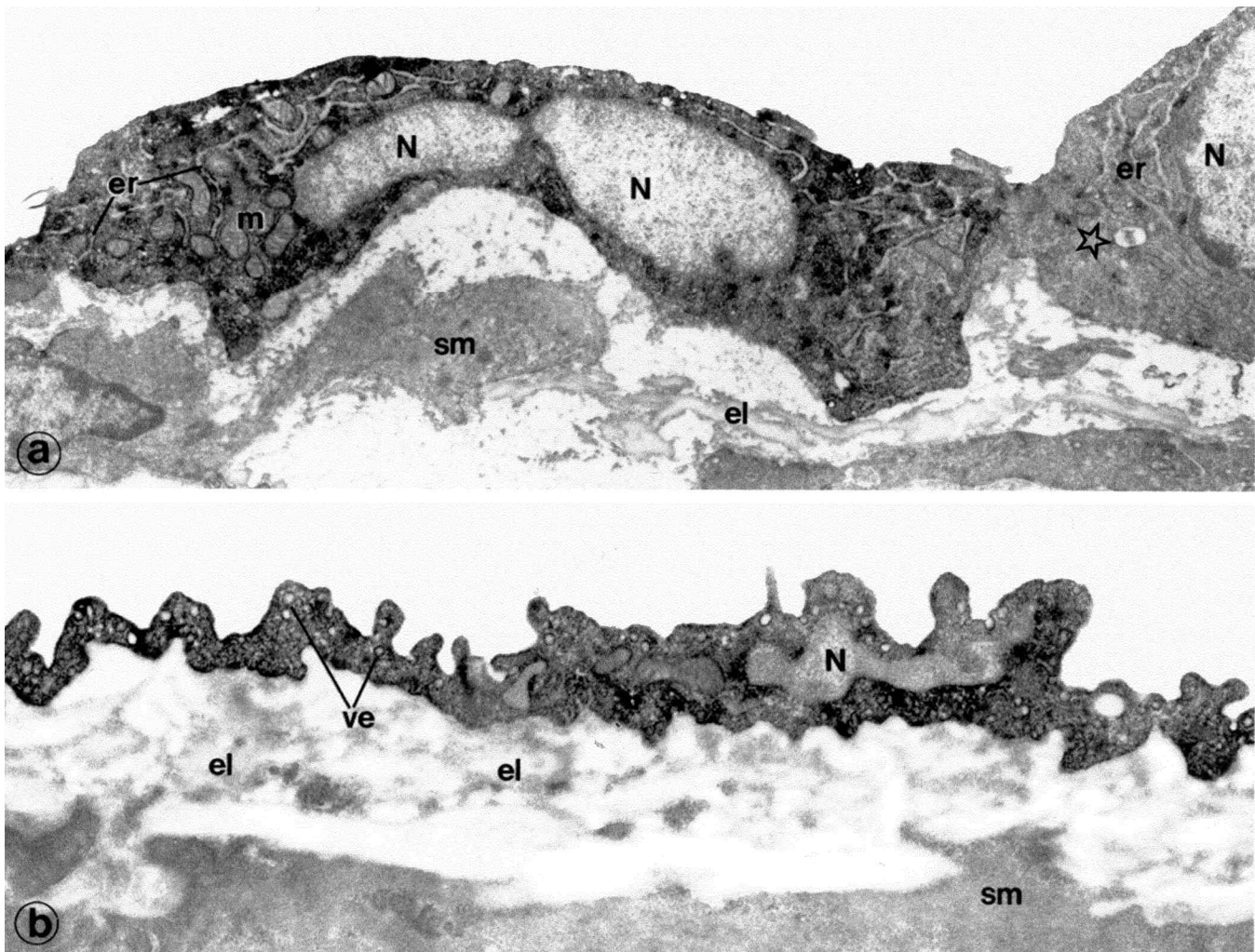


Fig. 3. Electron-immunocytochemical localisation of VP (PAP method) in the rat main pulmonary artery; comparison of newborn (a) and 2-year-old (b) animals. a. Note one VP-positive endothelial cell showing intense immunoprecipitate throughout the cytoplasm; the cytoplasm is rich in intracellular organelles including endoplasmic reticulum (er) and mitochondria (m). A fragment of slightly labelled endothelial cell is also seen (star). N: nucleus; el: elastic lamina; sm: smooth muscle. x 11,000. b. Note numerous cytoplasmic vesicles (ve) in VP-positive endothelium; endothelium appears irregularly shaped and flattened. x 22,000. For more information about immunocytochemistry of VP in endothelial cells of rat pulmonary artery see: Loesch et al., 1991.

Neuro-endothelial agents and receptors

Recently P2X receptor proteins have been revealed in vascular endothelial cells using immunocytochemical techniques, both at the electron and light microscope levels. An ultrastructural study of rat brain demonstrated the P2X₂ receptor subtype on endothelial cells of small cerebral vessels (Loesch and Burnstock, 2000b). A similar study of rat hypothalamus suggests that the cerebrovascular endothelium also contains P2X₆ receptor protein (Loesch, data unpublished). Immunohistochemical labelling at the light microscope level revealed the existence of P2X₂ and P2X₃ receptor subtypes in rat aortic and mesenteric endothelial cells, respectively (Hansen et al., 1999); the P2X₃ receptor subtype has been labelled in vascular endothelial cells of

the rat thymus (Glass et al., 2000), whilst endothelial cells that are immuno-positive for P2X₃, P2X₄ and P2X₇ receptors have been detected in rat thyroid blood vessels (Glass and Burnstock, 2001). The role of P2X receptors in endothelial cells has not yet been determined, although their participation in ionic trafficking, junction formation or modulation of the contractility of endothelial cells seems likely (Loesch and Burnstock, 2000b). In small cerebral vessels, abundant immunoreactivity to P2X receptors (P2X₂) has been detected at endothelial cell-cell contacts (Loesch and Burnstock, 2000b) suggesting the importance of these receptors for junction formation/function. Recent studies carried out on freshly harvested human vein endothelial

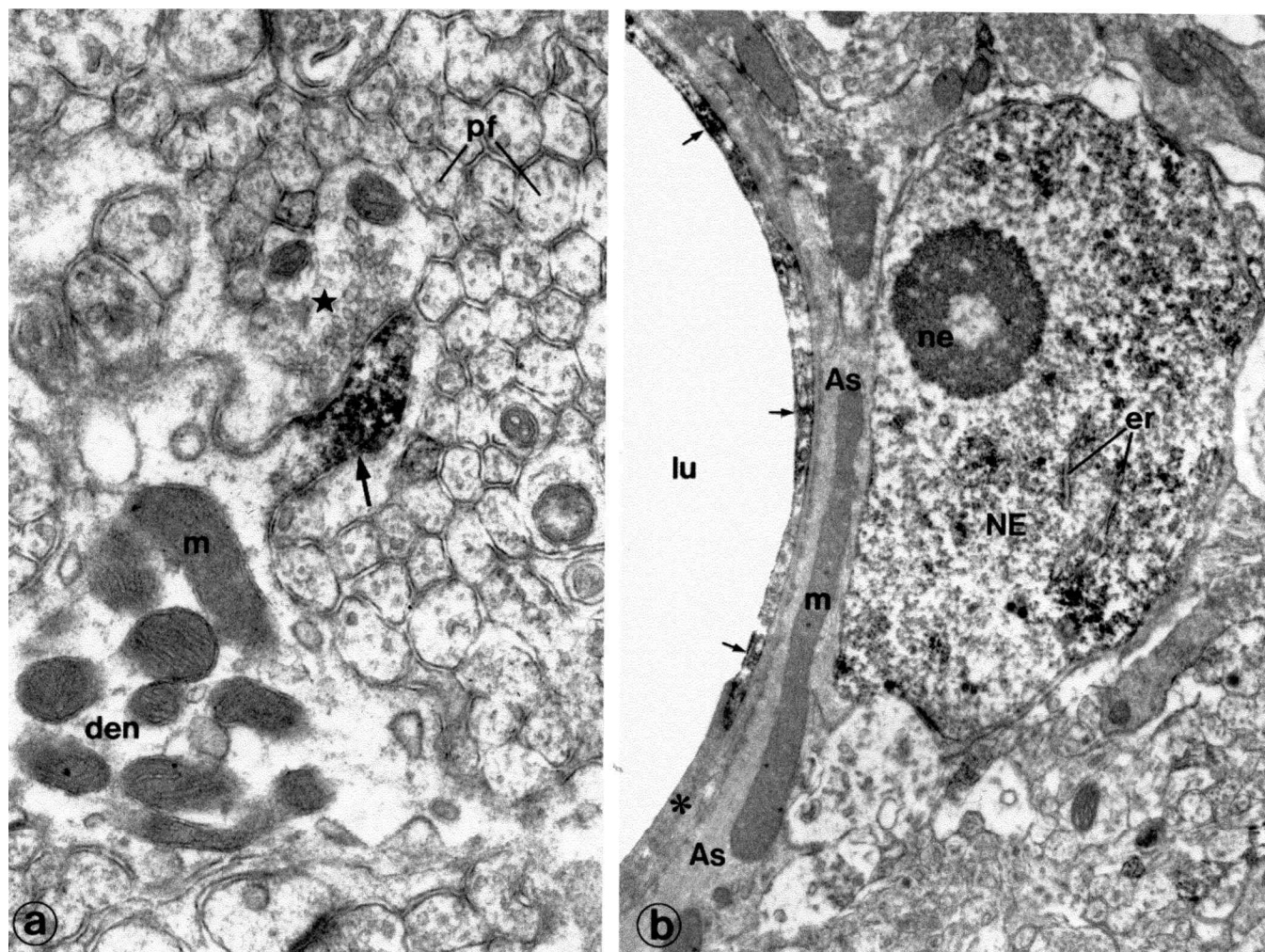


Fig. 4. Electron-immunocytochemical localisation (ABC method) of P2X₁ receptors in the cerebellum **(a)** and P2X₂ receptors in the hypothalamus **(b)** of the rat. **a.** Molecular layer of the cerebellum showing a dendrite (den) of a Purkinje cell displaying immunoreactivity for P2X₁, restricted to a spine (arrow); note a P2X₁-negative bouton (black star) en passant of parallel fibres of granule cells forming asymmetrical axo-dendritic synapse with the P2X₁-positive spine. pf: transversally sectioned parallel fibres, or small axons; m: mitochondria. x 42,000. **b.** The hypothalamic paraventricular nucleus displaying immunoreactivity for P2X₂ within the profile of magnocellular neurone (NE) and endothelial cells (arrows); immuno-negative endothelium can also be seen (asterisk). As: astrocytic processes; ne: nematosome/nucleolus-like body; lu: lumen of blood vessel. x 16,000. For more information about electron-immunocytochemistry of P2X₁ and P2X₂ receptors in rat brain see: Loesch and Burnstock, 1998b, 2000b; Loesch et al., 1999.

cells (HUVEC) by Glass and colleagues (Glass, Loesch, Bodin and Burnstock, data unpublished) suggest colocalisation of P2X receptors with adhesion molecules such as VE-cadherin, and the involvement of these receptors in junction formation. It should be stressed that P2X receptors in the CNS (Fig. 4a) are linked to ATP fast excitatory neurotransmission. Here, Fig. 4b demonstrates the presence of P2X₂ receptors on cerebrovascular endothelial cells, where the neighbouring neural profile also displays immunoreactivity for the receptor.

Conclusions

The functional significance of ET-1 innervation to cerebral arteries and the importance of endothelial cells displaying NPY, VP and P2X receptors, requires further investigation. These novel findings provide new directions for vascular research with potential contributions to our understanding of subtle processes/mechanisms underlying the endothelial and autonomic regulations of vascular tone and blood flow in physiological and pathological circumstances.

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