Endothelin-1 as a neuropeptide: neurotransmitter or neurovascular effects?

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Abstract Endothelin-1 (ET-1) is an endothelium-derived peptide that also possesses potent mitogenic activity. There is also a suggestion the ET-1 is a neuropeptide, based mainly on its histological identification in both the central and peripheral nervous system in a number of species, including man. A neuropeptide role for ET-1 is supported by studies showing a variety of effects caused following its administration into different regions of the brain and by application to peripheral nerves. In addition there are studies proposing that ET-1 is implicated in a number of neural circuits where its transmitter affects range from a role in pain and temperature control to its action on the hypothalamo-neurosecretory system. While the effect of ET-1 on nerve tissue is beyond doubt, its action on nerve blood flow is often ignored. Here, we review data generated in a number of species and using a variety of experimental models. Studies range from those showing the distribution of ET-1 and its receptors in nerve tissue to those describing numerous neurally-mediated effects of ET-1.

Keywords Endothelin · Neuropeptide · Neurovascular · Central nervous system · Peripheral nervous system

Abbreviations
2DG 2-deoxyglucose
ANS autonomic nervous system
Ax axon
At axon terminal
Av agranular vesicles
BVS blood vessels
CSF cerebrospinal fluid
CGRP calcitonin gene-related peptide
CNS central nervous system
Ds dendrite spine
ECE endothelin converting enzyme
[125I]-ET-1 iodinated endothelin-1
ET-1 endothelin-1
ET-2 endothelin-2
ET-3 endothelin-3
ETA endothelin A receptor
ETB endothelin B receptor
GABA γ-aminobutyric acid
Gv granular vesicles
ICV intracerebroventricular
Idcn intermediate dorsal cutaneous nerve
Lm lateral malleolus
LPS lipopolysaccharide
M mitochondrion
mRNA Messenger ribonucleic acid
NA noradrenaline
NO nitric oxide

Concise summary Apart from its vascular effects it has been suggested that endothelin-1 (ET-1) is a neuropeptide. This is based on the histological identification of ET-1 in nervous tissue with supporting data from functional studies. Here we review a number of studies into the potential neurotransmitter role of ET-1 and discuss its confounding neurovascular effects.
Introduction

Apart from its well-established vascular effects a number of studies suggest that endothelin-1 (ET-1) possesses neuropeptide properties via both ET_A and ET_B receptor subtypes. Perhaps one of the first publications in support of this proposition was the identification of positive immunostaining for ET-1 in neurones of the human spinal cord (Giaid et al. 1989). Furthermore, ultrastructural studies provided supporting evidence for a neurotransmitter role for ET-1 since ET-1-containing vesicles were identified in perivascular nerves of the rat basilar artery (Loesch et al. 1998).

Centrally-mediated actions of ET-1 were described within a few years of its identification based on blood pressure effects elicited in rats following intracerebroventricular administration (Macrae et al.1991a, 1993). A neurotransmitter role for ET-1 has also been proposed in the peripheral nervous system as topical application of this peptide was shown to alter nerve conduction (Zochodne et al.1992). Since publication of these early studies there may be some doubt regarding the transmitter properties of ET-1 as many reports over the last two decades suggest rather than being a neurotransmitter the actions of ET-1 are neurovascular. In this review, of the three endothelin isoforms (ET-1, ET-2 and ET-3) we focus mainly on ET-1 and discuss early histological data providing evidence that this peptide acts on sites within the central (CNS), autonomic (ANS) and peripheral (PNS) nervous systems and studies showing the consequences of application of ET-1 to these sites. Later reports describe neurovascular effects of ET-1 suggesting that many ‘neural’ actions attributed to this peptide may be secondary to reduced blood flow and ischaemia of nerve tissue.

Distribution of ET-1

Following the isolation and identification of ET-1 and the description of its vascular effects (Yanagisawa et al. 1988) it was not long before this vascular endothelium-derived factor was suggested to possess neuropeptide properties. Much of this was based on the initial immunohistochemical identification of ET-1 in nerve tissue from a number of species, including rat, mouse and human (Shinmi et al. 1989; Yoshizawa et al. 1990; Lee et al. 1990; Giaid et al. 1991; Franco-Cereceda et al. 1991; Nakamura et al. 1993; Gajkowska and Viron 1996). Regarding a potential neuropeptide role for ET-1 in vascular control, ET-1-positive varicosities have been identified in perivascular nerves of the basilar artery of the rat (Loesch et al. 1998) and capybara (Loesch et al. 2005) with a similar distribution in perivascular nerves/axons in human middle cerebral artery (Loesch et al. 2004, Loesch and Burnstock 2002; Mickey et al. 2002). In the periphery ET-1 has been shown to participate in pain-related processes (Khodorova et al. 2003, 2009).

ET-1 and the CNS

Distribution of ET-1 in the CNS

Apart from describing ET-1 distribution in brain of various experimental animals this peptide has also been identified in human brain (Naidoo et al.2004a, b). In addition, ET-1 mRNA and ET-1-like immunoreactivity has been described in human spinal cord and dorsal root ganglia with the authors concluding that ET-1 plays a part in neural transmission/modulation in addition to its vascular actions (Giaid et al. 1991).

Neurotransmitter actions of ET-1 in the CNS

The effects of drugs acting on the CNS may be studied in animals by administration into the cerebral ventricles. This technique was introduced over 50 years ago (Feldberg and Sherwood 1954) and, using such an approach, centrally-mediated effects of many drugs have been described. For example, the potential central inhibitory effect of endogenous opioid peptides on the adrenal medulla, have been suggested based on intracerebrovascular (ICV) administration of the opiate receptor antagonist, naloxone, in the cat (Dashwood and Feldberg 1979). In particular, ICV injection in rats, mostly into the lateral ventricles, is commonly used for screening of compounds with potential central cardiovascular activity. Here, the tip of the cannula is placed into the ‘liquor spaces’, often under asceptic conditions, using stereotacic guidance. Drugs administered by this route bypass the blood brain barrier, a structure that prevents many compounds reaching the brain when given systemically. Once injected into the cerebroventricular system compounds combine with the cerebrospinal fluid where they are able to act on many superficial brain structures. For example, the potential effects of a variety of neurotransmitters have been studied following
administration into the aqueduct, lateral and third ventricles, routes that target various superficial structures ‘adjacent’ to this compartment, including the periaqueductal gray matter and the hypothalamus (See Fig. 1).

ET-1 in the CNS: neuropeptide or cerebrovascular effects?

Patterns of brain activity may be studied by measuring local cerebral energy metabolism using 2-deoxyglucose (2DG) where altered uptake is suggested to be related to the local energy demands of nerve cells and therefore of nerve activity (Sokoloff 1978, 1984). This technique has been used to study neural ‘activity’ in many situations, particularly in relation to central cardiovascular control (Kostreva 1983). More recently the role of the cerebral microvasculature in supplying oxygen to ‘active’ nerve cells has been discussed with the proposal that 2DG reflects changes in blood flow rather than neural activity (Shepherd 2003). Although there is general acceptance that drugs acting when injected into the cerebral ventricles may affect neurotransmission it is clear that this may not necessarily be due to their neurotransmitter properties, more their action on the blood supply to various regions of the brain (Shepherd 2003). This suggestion was explored where the effect of ET-1 on cerebral blood flow was investigated following ICV administration. Experimental evidence was provided that ICV administration of ET-1 alters blood flow in the rat brainstem (Macrae et al. 1991b) with further data showing that this also occurs in vivo in cat cerebral resistance arterioles (Patel et al. 1996). Interestingly, where adventitial application of ET-1 and endothelin receptor antagonists was performed, ETA receptors were shown to be involved in constriction and ETB receptors with dilatation (Patel et al. 1996). These results are potentially important since in the peripheral vasculature ETB-receptor activation may also elicit vasoconstriction. If a similar distribution of vascular receptors occurs in man, selective ETA antagonist treatment may have therapeutic potential in certain cerebrovascular diseases. This suggestion is supported by a study using the mixed antagonist, bosentan, in the rat middle cerebral artery occlusion model. Here, the effects of bosentan on cortical perfusion was investigated using laser Doppler flowmetry where this compound failed to reveal a significant contribution of endogenous ET-1 in this model of focal ischaemic injury. The authors conclude that selective (not ‘mixed’) antagonists were needed to target ET-1-induced ischemia (McAuley et al. 1996).

A similar series of studies have been reported where ICV administration (into the lateral ventricle) of ET-1 in the rat was investigated. Initially, this group studied the potential central effect of ET-1 on systemic haemodynamics and regional blood flow using a radioactive microsphere technique. Whereas low doses (5 and 15 ng) of ICV ET-1 were ineffective, high doses (>45 ng) were shown to cause a transient rise, followed by a fall, in systemic blood pressure. These effects were accompanied by reduced blood flow to the brain, heart, kidneys, gastrointestinal tract, portal and musculo-skeletal systems. Pharmacological extension of these studies showed that these effects were mediated solely via ETA receptors (Rebello et al. 1995). In a subsequent study this group extended their findings by comparing the effects of ICV administration of ET-1 and ETA/ETB receptor-selective compounds. In addition to using
microspheres, sympathetic nerve activity was monitored. Here the authors showed that many of the effects of centrally administered ET-1, such as the fall in systemic blood pressure, were accompanied by a significant decrease in sympathetic nerve activity and that these effects were mediated via ETA receptors (Gulati et al. 1997; Kumar et al. 1997).

Macrae et al. (1991b) have shown that intracisternal administration of ET-1 in the normotensive rat caused a widespread and profound ischaemia of the caudal brainstem, a region involved in central cardiovascular control. ICV ET-1 injection produced a rise in mean arterial blood pressure and concomitant elevation of plasma noradrenaline and adrenaline levels. The authors conclude that ET-1 has the potential to induce cerebral ischaemia of pathological magnitude and wisely suggest that caution is needed when drawing conclusions from ICV drug administration as ET-1 is also capable of overriding cerebrovascular autoregulatory mechanisms.

The potential for ICV administered compounds to affect the cerebral circulation is evident from recent angiographic data produced in rats exhibiting drowsiness and coma caused by brain tumor (Hekmatpanah 2007). Here, a dense vascular network in normal rats was revealed using barium sulphate contrast medium (see Fig. 2). In control animals, magnified angiograms revealed perforating microvessels that penetrate the brain and, while their diameter is too small to be detected by routine cerebral angiography, India ink injections revealed small/terminal capillaries throughout the brain (Fig. 2). Interestingly, the author (Hekmatpanah 2007) describes infarcted and ischaemic changes to microvessels of rats with brain tumours similar to those that might be expected following administration of vasoconstrictors such as ET-1.

Further evidence for a predominant involvement of the ET\(_A\) receptor in the central cardiovascular effect of ET-1 was suggested where central administration of ET-1 was performed after stereotaxic placement of a needle tip into the periaqueductal gray area (D’Amico et al. 1996). These injections elicited a dose-dependent increase in mean arterial pressure but a fall in renal blood flow. Pretreatment with ET\(_A\)-selective antagonists reduced these effects but ET\(_B\) receptor blockade was ineffective. The potential role of ET\(_A\) receptors was supported by in vitro autoradiographic studies that showed dense \([^{125}\text{I}]\)-PD 151242 binding to many brain regions of the rat brain (including the periaqueductal gray area) and a paucity of ET\(_B\)-receptor binding sites (D’Amico et al. 1996). Again, the existence of the dense microvascular network in many brain regions, including the PAG, requires a potential vascular involvement of central ET-1 application to be considered (See Fig. 3).

A practical point that is ignored in many studies is the potential spread of what are believed ‘discrete’ injections and ‘drug targeting’. An injection volume of 1 µl will occupy an approximate area of 1 mm\(^3\). Such an area may encompass many brain nuclei that are likely to contain a dense network of cerebral vessels (Figs. 2 and 3).

In such a scenario caution is recommended if a neuropeptide action is suggested based on the effect(s) of centrally administered compounds.

Supporting evidence for a transmitter role for ET-1 has been described recently by Nabhen et al. (2009) where the co-existence of ET-1 and catecholaminergic neurones suggests that ET-1 may be a neuromodulator. Olfactory bulbs were obtained from normal rats and incubated in the presence/absence of ET-1, ET-3 and ET receptor antagonists.

![Fig. 2 Cerebral vessels. Left panel. Magnification angiography of normal rat brain using barium sulphate contrast medium. There is an abundant vascular supply with many penetrating microvessels in the cortex shown in the right panel following intra-arterial injection of India ink (black regions of a haemotoxylin and eosin stains section). Scale bar=5 mm for the left panel and 50 µm for the right panel. Modified from Hekmatpanah Surg Neurol 2007;67:564-71](image)
Tyrosine hydroxylase (TH) activity was determined, western blots performed and neuronal norepinephrine release measured. In the short term ET-1 and ET-3 both increase TH activity in vitro and this involved various mechanisms including increased calcium influx. This is said to be the first report showing an interaction between ET-1 and catecholaminergic transmission in olfactory bulbs and future studies are recommended to evaluate the relationship with the various other functions at this level of the brain.

CNS: ET-1 and the hypothalamo-neurosecretory system

While the endothelins and ET receptors have been detected in a number of structures throughout the CNS (Arai et al. 1990; Lee et al. 1990; Yoshizawa et al. 1990; Hemsen and Lundberg 1991; Nakamura et al. 1993; Horowitz et al. 1994; Uemura et al. 1994; Kurokawa et al. 1997; Yamada and Kurokawa 1998; Kurokawa et al. 2000), similar observations have been described in the endocrine hypothalamo-neurohypophysial secretory system that is responsible for delivering vasopressin and oxytocin to blood from neurosecretory axon terminals in the neurohypophysis (Krsmanović et al. 1991; Cao et al. 1993; Nakamura et al. 1993; Yasin et al. 1994; Mosqueda-Garcia et al. 1995; Kurokawa et al. 1997). Here we present selected examples of research relevant to the subject. A double immunogold co-labelling study by Nakamura et al. (1993) showed the colocalisation of immunoreactive ET-1 and vasopressin as well as ET-1 and oxytocin in subpopulations of neurohypophyseal axons of the rat. Also, co-expression of genes for ET-1 and oxytocin has been revealed in the rat during pregnancy (Horowitz et al. 1994); ET-1 mRNA has been shown to be increased significantly in the supraoptic and paraventricular nuclei from early to late gestation as has oxytocin gene expression as gestation advanced. Consequently, a neuroendocrine regulatory role for ET-1 in pregnancy has been suggested (Horowitz et al. 1994). A study of ET-1 gene transcription in rat supraoptic nucleus following injury showed the dynamism of ET-1-related mechanisms, namely time-associated changes in the expression of ET-1 mRNA and immunoreactivity to ET-1, suggesting an important role for ET-1 in neuroendocrine events following injury (Jiang et al. 2000).

Our own studies showed immunoreactive ET-1 within the rat hypothalamo-neurosecretory system, where ET-1 was detected in the cell bodies of endocrine neurons and in their processes containing neurosecretory granules (Mukherjee and Loesch 2002) (Fig. 4a). ET-1 was also detected in the axon terminals making axo-dendritic synapses; such terminals contain typical synaptic vesicles – mostly small agranular vesicles (Fig. 4b). Furthermore, these studies revealed a proportion of ET-1-positive endocrine neurons and their processes, as well as ET-1-positive axon terminals making axo-dendritic synapses that were also immunoreactive for neuronal nitric oxide synthase (nNOS) (Fig. 4a, b).

The significance of nNOS/ET-1 colocalisation may be difficult to explain but suggests that cotransmission and/or neuromodulation by ET-1 and NO occurs within these nerves. In general this supports the putative roles of NO and ET-1 in the CNS and PNS (Giaid et al. 1989; Snyder and Bredt 1992; Garthwaite and Boulton 1995; Knuepfer et al. 1994; Hoyle and Burnstock 1995; Lincoln et al. 1997; Rubino et al. 1999). Similarly, physiological roles for NO/nNOS and endothelins in hypothalamo-neurohypophysial neurosecretion may also occur (Yoshizawa et al. 1990; Yasin et al. 1993; Reid 1994; Rettori et al. 1997; Ng et al. 1999; Wang & Morris 1999; Costa et al. 2000; Kurokawa et al. 2000).
et al. 2000; Otukonyong et al. 2000). The axonal synaptic input at the level of the hypothalamic neurosecretory cell bodies of supraoptic and paraventricular nuclei is characterised by the presence of noradrenaline (NA), γ-aminobutyric acid (GABA) and glutamate (see Buller et al. 1996; Sperrlægh et al. 1998; Theodosis et al. 1998). Therefore it is likely that some of these axonal neurochemical inputs also include ET-1 and nNOS. Studies using hypothalamic explants from rat showed that NO inhibits the release of vasopressin (Yasin et al. 1993), while oxytocin may stimulate the release of NO from oxytocinergic neurones via NA-related mechanisms (Rettori et al. 1997). In rat, ET-1 converting enzyme (ECE; that converts its precursor big ET-1 to mature ET-1) is present in a subpopulation of neurosecretory neurons, including their axons, of the hypothalamo-neurohypophysial tract (Kurokawa et al. 2000). Histochemical evidence suggests that big ET-1 is secreted as a neurohormone from nerve terminals into the neurohypophysial blood vessels (Yamada and Kurokawa 1998). Hypothalamic endocrine nuclei display ET binding, in particular to ET<sub>A</sub> receptors (Kurokawa et al. 1997; Yamada and Kurokawa 1998). Strikingly, increases in circulating levels of ET-1 affect the hypothalamic endocrine nuclei producing predominantly excitatory effects on vasopressin- and oxytocin-neurones (Wall & Ferguson 1992). A recent study of the rat hypothalamus showed that ET-1 decreases neuronal NA uptake in the anterior hypothalamus and diminishes the uptake in the posterior hypothalamic region (Hope et al. 2008).

In addition to its neuroendocrine involvement the hypothalamus plays an important role in thermoregulation (Feldberg 1965). The potential role of ET-1 as a central mediator of fever has been studied where levels of big ET-1, the precursor of ET-1, were measured in cerebral spinal fluid (CSF) from rats after intravenous administration of lipopolysaccharide (LPS). Interestingly, big ET-1 levels in cisternal CSF fell in febrile rats versus controls, but levels were significantly increased in rats that had received ICV administration of the ETB antagonist, BQ788. Furthermore, intrahypothalamic injection of ET-1 caused a substantial and long lasting rise in rectal temperature. The authors conclude that LPS-induced fever in rat enhances ET-1 production in the brain (via measurement of big ET-1) and that ET-1 is an endogenous pyrogen (Fabricio et al. 2005).

**ET-1 and the PNS/ANS**

Cerebrovascular autonomic nerves

Although the endothelium is recognized as a major source of ET-1 in the vasculature it was reported that the autonomic perivascular nerves in rat basilar artery are immunopositive for ET-1 with 36% of the axon profiles (varicosities and intervaricosities) in the adventitia of the rat basilar artery observed at the electron microscopic level being immunolabelled for ET-1 (Loesch et al. 1998). Axon

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Fig. 4 Neuropil of supraoptic nucleus of the hypothalamus double-labelled for nNOS (immunoprecipitate - long arrows) and ET-1 (immunogold-silver grains – short arrows). a A fragment of a double-labelled dendrite (likely to be a primary or varicose dendrite from a bipolar magnocellular neurone) shows abundance of neurosecretory granules (nsg), of which some are NOS-positive (long arrows); short arrows point to immunoreactivity for ET-1. Note unlabelled axon profile (Ax) making synapse on the double-labelled dendrite. b A double-labelled axon terminal (At) making asymmetric synapse with unlabelled dendrite spine (ds). The axon terminal contains numerous small agranular synaptic vesicles, membrane of which is labelled with nNOS immunoprecipitate; co-localised immunogold-silver grains of ET-1 labelling (arrows) are also seen. Note that an adjacent axon profile (Ax) containing small agranular synaptic vesicles is unlabelled; m-mitochondria. Bars: 0.5 μm. Affinity-purified rabbit polyclonal nNOS antibody (SC-1025, Santa Cruz, USA), which does not cross react with NOS2 and NOS3 was used at 0.8 μg/ml in the preembedding ExtrAvidin immunocytotoxic method. The rabbit polyclonal ET-1 antibody to human/porcine ET-1 (Sigma, Poole, UK), which does not cross-react with big ET but may with ET-2 and ET-3 was used at 1:1,000 in the preembedding immunogold-silver labelling method as the second immunolabelling. Images A and B are modified from Mukherjee and Loesch, Histochem J 2002, 34:181-187 [Kluwer Academic Publishers], which is kindly acknowledged.

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varicosities of these ET-1-positive nerve fibres displayed small agranular vesicles (~ 42 nm) and large granular vesicles (~92 nm) with ET-1-labelled cores (Fig. 5a). Subsequent studies focusing on the basilar artery of spontaneously hypertensive rats showed an increase in ET-1-positive perivascular axons in such conditions, although these axons usually displayed structural abnormalities (Fig. 5b) (Milner et al. 2000a). Combined immunohistochemical, in situ hybridisation and sensory and sympathetic denervation studies revealed that the ET-1-containing cerebrovascular nerves originate from the sensory and sympathetic components of the trigeminal (TG) and superior cervical (SCG) ganglia and that the ET-1 in a proportion of the TG neurons is co-localised with sensory substance P (Milner et al. 2000a, b). This suggests that the sensory TG is the more likely source of ET-1-containing perivascular nerves to cerebral arteries than the sympathetic SCG (Milner et al. 2000b).

Since this denervation study suggests that ET-1-positive perivascular nerves of large cerebral arteries may be the projections of primary afferent sensory neurons of the TG this raises the possibility that ET-1 may co-exist with other sensory transmitters such as the neuropeptides substance P and calcitonin gene-related peptide (CGRP), known to be present in TG sensory nerves projecting to large
cerebral arteries (Suzuki et al. 1989). Our own electron-immunocytochemical study in capybara, the largest rodent, revealed that cerebrovascular nerves contain both ET\textsubscript{A} and ET\textsubscript{B} receptors (Fig. 6), suggesting that neurotransmission involving ET-1 occurs in cerebral arteries (Loesch et al. 2005). Patients with cerebrovascular disease exhibit an increased expression of ET\textsubscript{B} receptors (Hansen-Schwartz 2004; Hansen-Schwartz et al. 2002) and the expression of ET\textsubscript{B} receptors has also been described in perivascular nerves of microvessels of the sensorimotor cortex and hippocampus after brain trauma (Kallakuri et al. 2007) where it is suggested that ET\textsubscript{B} receptor expression is linked with both vasoconstriction and attenuation of ET-1 synthesis and availability.

A number of studies describe in detail a variety of pharmacological and physiological effects linked with ET-1 and the adventitial region of cerebral vessels. These include experiments with the injections of ET-1 to the cisterna and vertebral artery (Mima et al. 1989) as well as using well-established cerebral artery occlusion models, mostly of the middle cerebral artery in the rat or mouse simulating stroke and ischemic conditions in adjacent brain tissue (Ahn et al. 2002; Callaway et al. 2003; Gresle et al. 2006; Gupta et al. 2006; Miller et al. 2006). In these models, localised microinjection of ET-1 affects the perivascular but not the luminal compartment of the middle cerebral artery causing vasoconstriction. ET-1 was proposed as a major cause of cerebral vasospasm in subarachnoid hemorrhage (SAH), and having stronger vasoconstrictor effects than ET-2 and ET-3 (Zimmermann and Seifert 1998). This phenomenon (and/or similar ones) may also shed some light on the possible involvement of ET-1-containing cerebrovascular nerves in vasoconstriction where stimulation of such nerves releases neural ET-1 resulting in vasoconstriction of the cerebral artery that they innervate. This hypothesis might also be extended to other vascular beds including the rat femoral artery, where sustained arterial constriction following prolonged exposure to perivascular ET-1 was reported (Ahn et al. 2002).

In addition to adventitial cerebrovascular nerves the adventitial Schwann cells of the vessel wall are also implicated in certain mechanisms related to the action of ET-1. For example, in the adventitia of the middle cerebral artery of man a close apposition of ET-1-positive Schwann cells and ET-1 negative axon varicosities have been observed suggesting an interaction between these cells (Loesch et al. 2004). There is a suggestion that glial cells, such as astrocytes, are the main local source of ET-1 in the subarachnoid space following hemorrhage and cerebral ischemia (Pluta et al. 1997). Therefore, the possibility exists that ET-1 derived from glial cells, rather than from the cerebral vascular endothelium, is the likely participant

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![Fig. 7 Endothelin and its receptors on human sural nerve.](https://example.com/f7)

**Fig. 7** Endothelin and its receptors on human sural nerve. a Location of human sural nerve: (From Aktan Ikiz et al. 2005) sn: sural nerve, lm: lateral malleolus, icdn: intermediate dorsal cutaneous nerve. b Distribution of ET-1, ET\textsubscript{A} and ET\textsubscript{B} receptors on human sural nerve sections revealed by in vitro autoradiography using radiation-sensitive film (low resolution). c Microscopic localisation of ET-1 on a section of human sural nerve. Left panel is a dark-field illumination, ‘high resolution’ autoradiograph of a section incubated in \(^{125}\text{I}\)-labelled ET-1 and detected using nuclear emulsion where white grains show receptor binding sites. Right panel is the haemotoxylin and eosin stained tissue. There is strong binding to the epineurial vessels (BVS), perineurium (arrowheads) and vasa nervorum (small cell clusters within the nerve fascicles surrounded by the perineurium). Scale bars=2 mm for autoradiographs in B and 50 \(\mu\)m for C (B and C modified from Dashwood and Thomas 1997)
in certain cerebrovascular disorders (Pluta et al. 1997). It is also recognised that ET-1 plays a role in Schwann cells during nerve development and degeneration and that ET-1 can stimulate or inhibit the release of neurotransmitter(s) from sympathetic neurons (Damon 1999; Barti-Mattera et al. 2001; Pomoni and Almhanna 1994). A later report, using a diabetic rat model, showed that endogenous ET-1 plays a potential role in diabetic neuropathy via an action on nerve blood perfusion (Stevens and Tomlinson 1995). Here, using streptozotocin-induced diabetic rats, after 6 weeks of untreated diabetes, infusion of an ETA receptor antagonist caused a significant increase in sciatic nerve conduction whereas in non-diabetic rats it was ineffective. In diabetic rats sciatic nutritive endoneurial vessels and subsequent ischaemia may have a pronounced effect on nerve function. Thus, certain neurotransmitter actions may be secondary to ET-1-induced constriction of the endoneurial vessels and vasa nervorum. For example, local application of ET-1 to rat sciatic nerve causes reduced axonal conduction, due to a localised effect on epineurial microvessels and subsequent ischaemia of the nerve (Cameron et al. 1994). A later report, using a diabetic rat model, showed that endogenous ET-1 plays a potential role in diabetic neuropathy via an action on nerve blood perfusion (Stevens and Tomlinson 1995). Here, using streptozotocin-induced diabetic rats, after 6 weeks of untreated diabetes, infusion of an ETA receptor antagonist caused a significant increase in sciatic nerve conduction whereas in non-diabetic rats it was ineffective. In diabetic rats sciatic nutritive endoneurial blood flow was reduced, an effect that was significantly ameliorated by ETA receptor antagonist treatment. In non-diabetic rats antagonist treatment had no effect on blood flow, suggesting that ET-1 is not involved in the control of nerve blood flow in normal rats but makes a major contribution to the perfusion deficit in experimental diabetes raising the possibility that ET-1 receptor blockade may have therapeutic potential in diabetic patients (Stevens and Tomlinson 1995). These observations may be relevant to data obtained in human sural nerve biopsies where, using in vitro receptor autoradiography, [125I]-ET-1 binding was associated with various structures, in particular the perineurium, endoneurial vessels and vasa nervorum (Fig. 7; Dashwood and Thomas 1997). This binding was reduced (competitively) when sural nerve sections were incubated in the presence of the mixed ET receptor antagonist, bosentan, providing evidence that (like the diabetic rat model) the ‘neuropeptide’ role of ET-1 may be mediated via ‘neurovascular’ effects where altered nerve conduction is associated with an ET-1-induced reduction in nerve blood flow and subsequent nerve ischaemia (Dashwood and Thomas 1997).

Conclusions

Apart from the well-established vasoactive and proliferative effects of the endothelins, in particular ET-1, it is suggested that this peptide also possesses neurotransmitter activity. While a neuropeptide role for ET-1 is supported by its histological identification in both the central and peripheral nervous systems caution is needed in interpretation of results since certain ‘neural’ effects of this peptide may be due to its potent neurovascular action. However, the therapeutic potential of endothelin receptor subtype-selective antagonists in a number of neural conditions remains regardless of their site of action.

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