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Endothelin-1 and endothelin receptors in the basilar artery of the capybara

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Summary

Little is known about cerebral vasculature of capybara, which seems may serve as a natural model of studying changes in cerebral circulation due to internal carotid artery atrophy at animal sexual maturation. This is the first study of the light- and electron-immunocytochemical localisation of endothelin-1 (ET-1) and ET_A and ET_B endothelin receptors in the basilar artery of capybaras (6 to 12-month-old females and males) using an ExtrAvidin detection method. All animals examined showed similar patterns of immunoreactivity. Immunoreactivity for ET-1 was detected in the endothelium and adventitial fibroblasts, whilst immunoreactivity for ET_A and ET_B receptors was present in the endothelium, vascular smooth muscle, perivascular nerves and fibroblasts. In endothelial cells immunoreactivity to ET-1 was pronounced in the cytoplasm or on the granular endoplasmic reticulum. Similar patterns of immunolabelling were observed for ET_A and ET_B receptors, though cytoplasmic location of clusters of immunoprecipitate seems dominant. These results suggest that the endothelin system is present throughout the wall of the basilar artery of capybara.

Introduction

The capybara (Herrera & Macdonald 1984) is the world's largest rodent weighing 30–70 kg. The anatomical and physiological details of this animal are mostly unknown including that of the cerebral vascular bed. However, studies by Reckziegel and colleagues (2001) reported on the macro-anatomical details of the brain vascularisation in this animal, including the contribution of the basilar artery. According to this study, the basilar artery in capybara is a central component of the vertebro-basilar system supplying blood to the brain. The role of this artery seems particularly important after regression of the internal carotid artery in mature capybaras – this concerns both males and females (Reckziegel *et al.* 2001). This fact makes study of this animal of particular interest with regard to the cerebral circulation, with possible relevance to clinical medicine. The possibility arose from a study by Islam and colleagues (Islam *et al.* 2004) that the cerebral vasculature of the capybara could undergo remodelling during regression of the internal carotid artery.

The present study focuses on endothelin (ET-1) and endothelin receptors: ET_A and ET_B in capybara basilar artery, which has not previously been examined. ET-1, which is generally recognised as a powerful vasoconstrictor (Yanagisawa *et al.* 1988), belongs to family of ET peptides (ET-1, ET-2, and ET-3), expressed by a variety of cell types including endothelial cells (Yanagisawa *et al.* 1988), macrophages (Ehrenreich *et al.* 1990), astrocytes (MacCumber *et al.* 1990), and neurones (Giaid *et al.* 1989). ET-1 has previously been demonstrated in the cerebral vascular bed of mammals including rat, rabbit and man, where it was mainly localised to cerebrovascular endothelial cells (Loesch *et al.* 1993, 1998, Gorelova *et al.* 1996, Loesch & Burnstock 1996a, b, 1998, Shochina *et al.* 1997). ET-1 has also been shown in cerebrovascular autonomic nerves to large cerebral arteries of rat and man (Loesch *et al.* 1998, Milner *et al.* 2000a, Loesch & Burnstock 2002, Loesch 2003). Studies on sensory and sympathetic denervation of the rat suggested that sensory autonomic nerves projecting from the trigeminal ganglion are a more likely source of ET-1-containing perivascular nerves in cerebral arteries

than sympathetic nerves from the superior cervical ganglion (Milner *et al.* 2000b). Pharmacological evidence suggests that ET-1 (and also ET-2) is a powerful vasoconstrictor of the cerebral vascular bed (Kobayashi *et al.* 1990), most likely due to the presence of ET_A receptors (Adner *et al.* 1993, Hirose *et al.* 1995, see Zimmermann & Seifert 1998). Studies of cerebral arteries of various mammals showed that alongside contractile ET_A receptors, dilatory ET_B receptors may be present (Feger *et al.* 1994, Goadsby *et al.* 1996, Yakubu & Leffler 1996). The contractile ET_A and dilatory ET_B receptors and their mRNAs have also been reported in human cerebral arteries; mRNAs were detected both in the presence and absence of the endothelium (Nilsson *et al.* 1997).

The aim of this study was to examine the distribution of the vasoconstrictor ET-1 (Yanagisawa *et al.* 1988) and ET_A and ET_B receptors in the capybara basilar artery, using immunocytochemical and electron microscopic techniques of detection.

Materials and methods

Animals and immunocytochemistry

Five 6-month-old ('young'; 3 females and 2 males) and five 12-month-old ('mature'; 2 females and 3 males) capybaras were used in this study. The animals' basilar arteries were obtained from the Profauna Farm in São Paulo, Brazil, under a licence granted by the Brazilian Institute of the Environment – IBAMA (1-35-93-0848-0). Animals were killed by chemical euthanasia using an overdose of pentobarbitone, which was administered intravenously at 80 mg/kg. The jugular vein was then opened to allow perfusion-fixation (~500 ml of fixative) of the cerebral vasculature using a cannula inserted into the common carotid artery. The fixative consisted of 4% paraformaldehyde and 0.2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The basilar artery (from each animal) was dissected out and placed in the same fixative overnight at 4 °C. It was then transferred to the stock buffer (0.1 M phosphate buffer containing 0.1% sodium azide) and afterwards to 0.1 M TRIS buffered-saline (TBS), at pH 7.6. Cross-sections of the artery ~100 μm thick were cut using a vibratome, collected in TBS and immunoprocessed (at room temperature) for electron microscopy. In brief: sections were (a) exposed to 0.3% hydrogen peroxide in 33% methanol for 45 min (in order to block endogenous peroxidases), (b) washed in TBS, (c) placed for 1 h in 10% heat-treated normal goat serum (Nordic Immunology, Tilburg, the Netherlands) (for ET-1 detection) or in 10% heat-treated normal horse serum (Jackson ImmunoResearch Laboratories, West-Grave, PA, USA) (for ET_A and ET_B receptors detection), (d) washed in TBS, (e) incubated for 24 h at 8 °C with a monoclonal antibody to ET-1 (diluted 1:1000 in TBS containing 10% heat-treated normal goat serum and 0.1% sodium azide) or polyclonal antibodies to ET_A and ET_B (diluted 1:400 in TBS containing 10% heat-treated normal horse serum and 0.1% sodium azide), (f) washed in TBS, (g) for ET-1 labelling, incubated for 12 h at 8 °C with a biotin-conjugated goat anti-mouse IgG serum (Jackson) (diluted 1:500 in TBS containing 1% heat-treated normal goat serum and 0.1% sodium azide) or for ET_A and ET_B labelling, with a biotin-conjugated donkey anti-rabbit IgG serum (Jackson) (diluted 1:500 in TBS containing 1% heat-treated normal horse serum and 0.1% sodium azide), (h) washed in TBS, (i) exposed for 5 h at 8 °C to ExtrAvidin-horseradish peroxidase conjugate (Sigma, Poole, UK) diluted 1:1500 in TBS, and then (j) washed in TBS. The immunoreactivity was visualised with diaminobenzidine (Sigma). After extensive rinsing in TBS and distilled water, sections were placed in 1% osmium tetroxide for 1 h (at 4 °C), washed in 0.1 M sodium cacodylate buffer, dehydrated in ethanol and propylene oxide and flat embedded in Araldite and polymerised. Ultrathin sections were stained with uranyl acetate and lead citrate, and subsequently examined and photographed with a JEOL-1010 transmission electron microscope (TEM). Semithin sections (1.5 μm) were also cut. These were stained with toluidine blue and then examined using a Zeiss Axioplan microscope (Zeiss, Germany), and optical images obtained with Leica DC200 digital camera.

Immunocytochemical controls

Mouse monoclonal ET-1 antibody (MCE-6901-01, clone IC4, isotype IgG_1) to human ET-1 was manufactured and characterised by Peninsula Laboratories – Bachem UK Limited, St Helens, England. The antibody specifically recognises ET-1 (in conjunction with anti-C-terminal has a sensitivity of 0.06 pmol/l) (Peninsula Laboratories). A successful labelling of cerebral vessels with this antibody has been shown (e.g., Mickey *et al.* 2002). In the present study, the routine omission of the ET-1 antibody and IgG steps, independently, resulted in lack of immunolabelling. Similar controls were performed for immunolabelling with ET_A and ET_B rabbit polyclonal antibodies (Alomone Labs, Jerusalem, Israel). The anti-ET_A receptor antibody (AER-001) recognises intracellular (C-terminus) epitope corresponding to amino acid residues 413–426 of rat ET_A peptide (Accession P26684), whilst the anti-ET_B receptor antibody (AER-002) recognises intracellular (i3 loop) epitope corresponding to residues 298–314 of rat ET_B peptide (Accession P21451). Both antibodies were affinity purified on immobilized antigens (Alomone Labs). It has also been demonstrated that the ET_A receptor shows homology in a number of

species including human, swine, bovine, rat and chicken, whilst the homology of ET_B receptor appears identical in all vertebrates studied (Alomone Labs).

Results

All animals examined showed a well-structured basilar artery comprising of intimal endothelial cells, medial vascular smooth cells, adventitial autonomic perivascular nerves and fibroblasts (see previous study from our labs – Islam *et al*. 2004). The application of immunocytochemistry identified the antigenic sites of ET-1 and ET_A and ET_B receptors in the basilar artery. This localisation was clearly visible both at the light (Figure 1) and electron microscopic (Figures 2–6) levels. No immunoreactivity was observed in the immunocytochemical control preparations (Figure 1a). There were no clear immunocytochemical differences observed between the age and gender of animals; the following description applies to all animals examined.

Light microscopy

Light microscopic immunocytochemistry revealed positive ET-1 immunolabelling in most of the intimal endothelial cells observed (Figure 1b). The immunoreactivity for ET-1 was also observed within the adventitia, where it labelled fibroblast-like cell profiles. This was particularly noticeable in 12-m-old capybaras (Figure 1c). Light microscopy revealed a similar

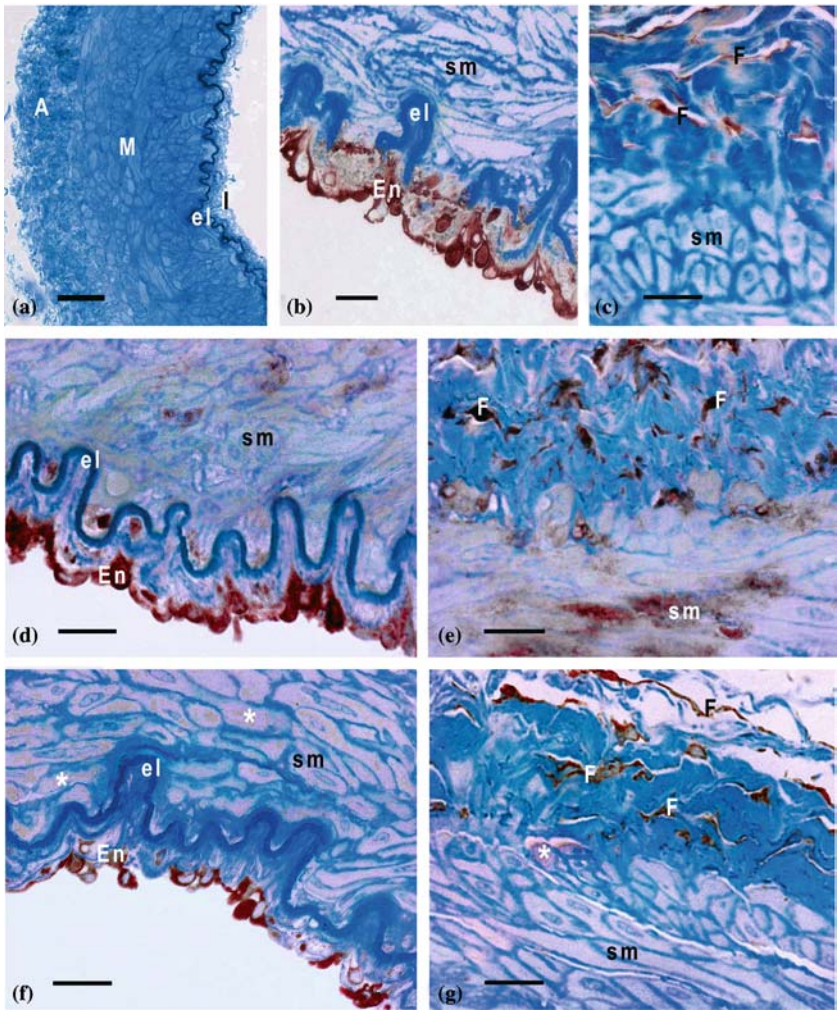

Figure 1. (a–g) Light microscopy of semithin section of capybara basilar artery processed for the controls (a), and immunolabelled (orange-dark brown label) for ET-1 (b, c), ET_A (d, e) and ET_B (f, g) - sections were counterstained with toluidine blue. (a) This image is an example of a control basilar artery showing no immunolabelling when the primary antibody step was omitted. A-adventitia, M-media, I-intima, el-internal elastic lamina. In (b) and (c) note the endothelial cells (En) and adventitial fibroblasts (F) are immunoreactive for ET-1, respectively; smooth muscle cells (sm) are ET-1-negative. In (d) note that the endothelial cells are immunoreactive for ET_A receptors; (e) shows that the immunoreactivity for ET_A receptors also labels medial vascular smooth muscle cells and adventitial fibroblasts. (f) and (g) These sections of basilar artery demonstrate that immunoreactivity for ET_B receptors is present both in the endothelium and adventitial fibroblasts; medial smooth muscle cells show diffuse immunoreactivity (asterisks). [(a, e, f) are from 6-month-old females; (b, d, g) are from 6-month-old males; (c) is from a 12-month-old female]. Bars: (a) = 50 μm, (b–g) = 20 μm.

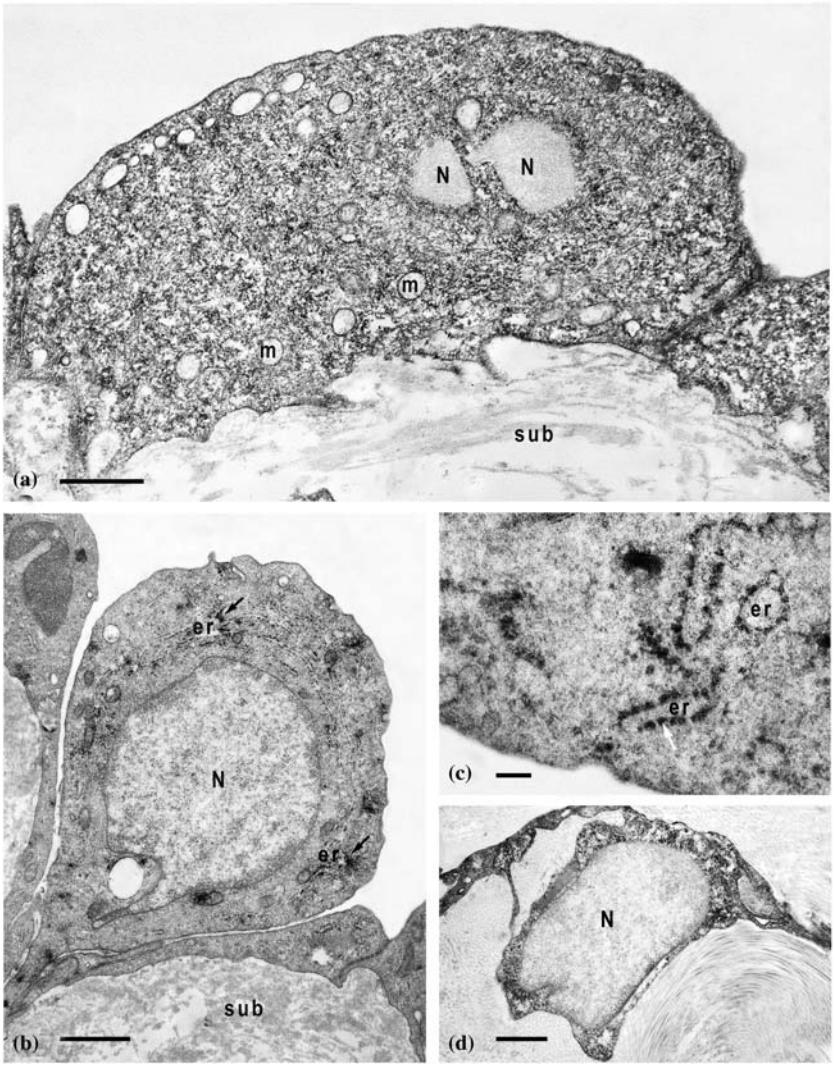

Figure 2. (a–c) Electron microscopy of endothelium of capybara basilar artery immunolabelled (black precipitate) for ET-1. (a) An example of endothelial cells displaying cytoplasmic localisation of immunoreactivity for ET-1. Unlabelled nucleus (N) and mitochondria (m) can be seen. sub-subendothelial connective tissue. (b) An example of endothelial cell demonstrating that the clustered immunoreactivity for ET-1 (arrows) is close to or associated with the granular endoplasmic reticulum (er). (c) High magnification fragment of an endothelial cell showing that the ET-1-immunoprecipitate (arrow) is associated with the cytosolic sites of the cisterns of the granular endoplasmic reticulum. (d) An example of ET-1-positive adventitial fibroblast displaying cytoplasmic labelling for ET-1. [(a) is from a 6-month-old female; (b, c) are from 12-month-old males; (d) is from a 12-month-old-female]. Bars: (a, b, d) = 1 μm, (c) = 100 nm.

distribution of positive immunoreactivity for ET_A (Figure 1d, e) and ET_B (Figure 1f, g) receptors that was localised to endothelial cells, vascular smooth muscle cells, and cells located in the adventitia. Vascular smooth muscle cells showed more prominent immunolabelling for ET_A (Figure 1e) than ET_B (Figure 1g) receptors; the latter labelling usually appeared as diffuse staining at the light microscope level.

Electron microscopy

ET-1 (Figure 2a, b)

At the electron microscopic level it was apparent that the ET-1-positive endothelial cells displayed immunoreactivity localised mostly throughout the cell cytoplasm (Figure 2a). These cells were usually rich in cytoplasmic organelles and structures. In some endothelial cells of 12-month-old animals, immunoreactivity for ET-1 predominated on the cytoplasmic site of granular endoplasmic reticulum or appeared as small clusters in the cytoplasm (Figure 2b, c). Most of the fibroblasts in the adventitia displayed immunoreactivity for ET-1 (Figure 2d), which exhibited a cytoplasmic localisation.

ET_A and ET_B receptors (Figures 3–6)

Electron microscopic examination of basilar artery revealed immunoreactivity for both receptors in the endothelium of the intima (Figure 3), vascular smooth muscle of the media (Figure 4) as well as perivascular nerves including axon varicosities (Figure 5) and adventitial fibroblasts (Figure 6).

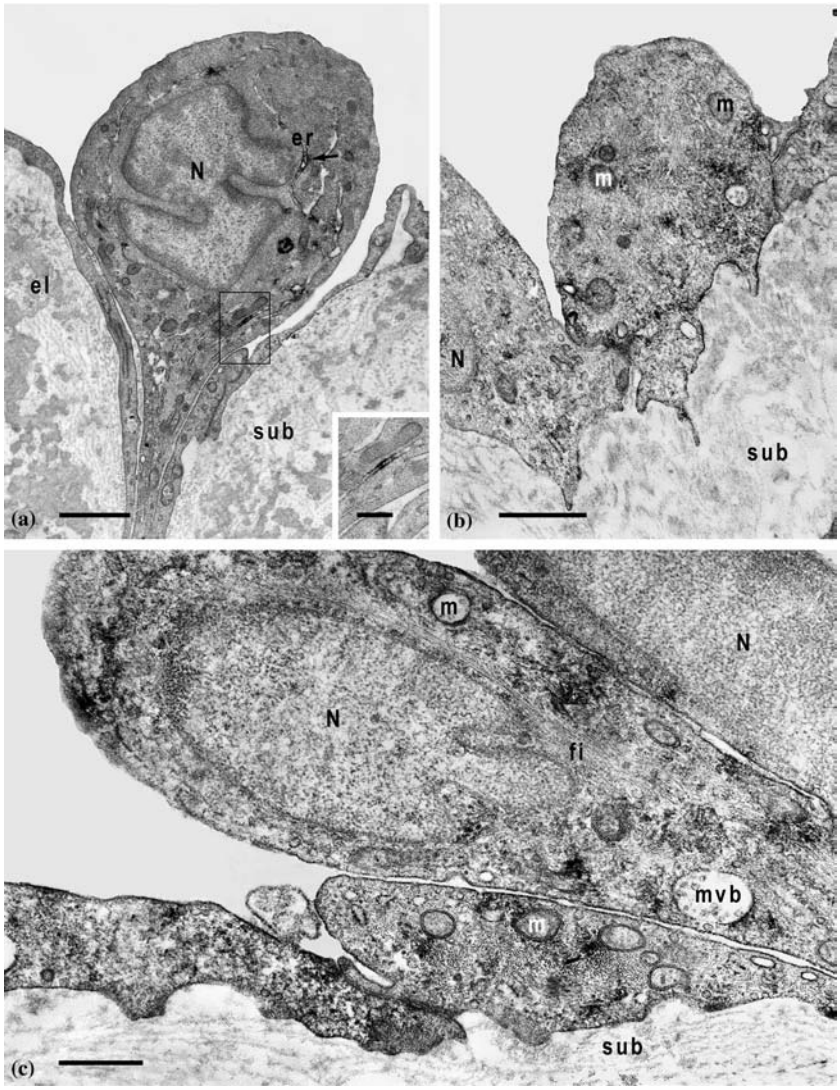

Figure 3. (a–c) Electron microscopy of endothelium of capybara basilar artery immunolabelled (black precipitate) for ET_A (a, b) and ET_B (c) receptors. (a) Note the localisation of immunoreactivity for ET_A (arrows) in the cisterns of the granular endoplasmic reticulum (er). N-nucleus, sub-subendothelial matrix. Inset is an enlargement of the area of the endothelial cell indicated in a box. (b) Note 'patchy' cytoplasmic pattern of immunoreactivity for ET_A. m-mitochondria. (c) This image of endothelial cells shows that the immunoreactivity for ET_B has a patchy cytoplasmic localisation, similar to that observed in (b). mvb-multivesicular body, fi-cytoplasmic filaments. [(a) is from a 6-month-old male; (b, c) are from 12-month-old females]. Bars: (a, b) = 1 μm, (c) = 0.5 μm, inset = 250 nm.

Endothelium. The immunoreactivity for ET_A and ET_B receptors was localised within the cisterns of endoplasmic reticulum (Figure 3a) or more frequently in the cytoplasm of the cells, where it appeared distributed in a 'patchy' fashion (Figure 3b, c). However, the nuclei of endothelial cells were unlabelled.

Vascular smooth muscle cells. Smooth muscle cells showed intimate immunoreactivity for ET_A and ET_B receptors. Immunoreactivity was distributed as various sized clusters located in the sarcoplasm; no association of the labelling with any particular intracellular structure was observed (Figure 4a, b). However, the immunoreactivity for ET_B receptors was also detected on the sarcolemma (Figure 4b).

Perivascular nerves. Immunoreactivity for ET_A and ET_B receptors was associated with perivascular nerves, both their axon profiles and accompanied Schwann cells (Figure 5a, b). In axon varicosities positive for ET_A, the immunolabelling was associated with granular synaptic vesicles (Figure 5a); in varicosities positive for ET_B most of the immunolabelling was localised in the axoplasm region close to the contact with the accompanied Schwann cell (Figure 5b). In some nerve profiles, immunoreactivity for ET_B was localised in the Schwann cells only, whilst associated axon varicosities were negative for ET_B receptors (Figure 5c).

Fibroblasts. Immunoreactivity for ET_A and ET_B receptors was found in the fibroblasts where immunostain-

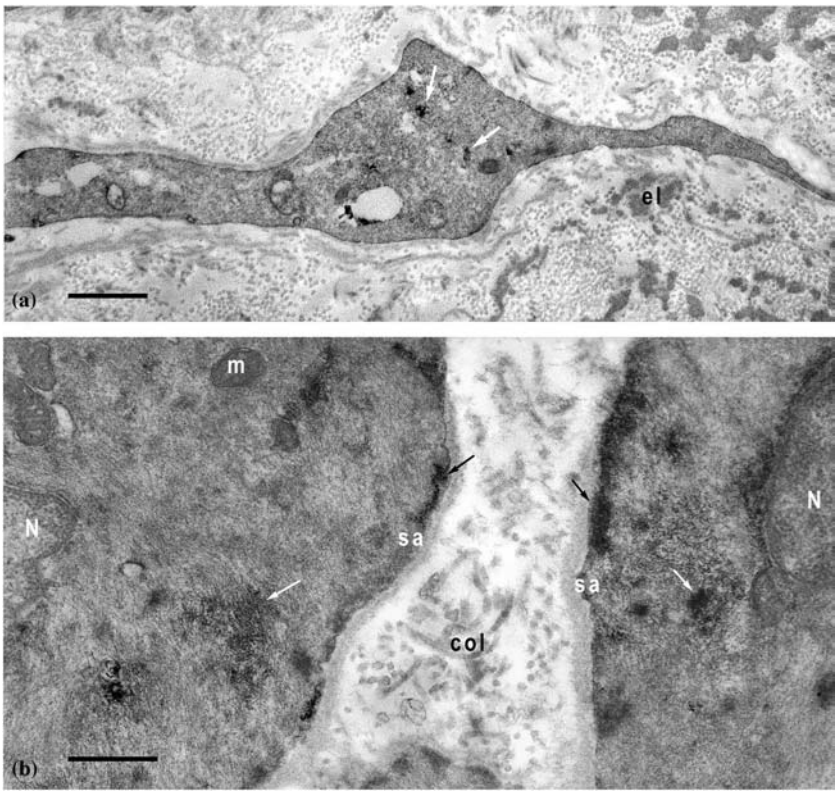

Figure 4. (a, b) Electron microscopy of vascular smooth muscle cells of capybara basilar artery immunolabelled (black precipitate) for ET_A (a) and ET_B (b) receptors. Note that immunoreactivity for ET_A and ET_B receptors is distributed as small clusters (white arrows) located in the sarcoplasm; in (b) an association of the labelling for ET_B receptors with the sarcolemma (sa) can also be seen (black arrows). N-nucleus, el-elastin, col-collagen. [(a) and (b) are from a 6-month-old male and female, respectively]. Bars: (a) = 1 μm, (b) = 0.5 μm.

ing was localised in small patches of the cytoplasm, and on the strands of the granular endoplasmic reticulum. (Figure 6a, b).

Discussion

The structural details of the basilar artery of capybara have been previously reported (Islam *et al.* 2004). The present study revealed the presence of immunoreactive ET-1 peptide and ET_A and ET_B receptors in the basilar artery of this species that was localised in the endothelium, smooth muscle cells, perivascular nerves, and fibroblasts.

ET-1

The immunoreactivity of ET-1 within endothelial cells of both young and mature capybaras was seen in the cell cytoplasm and on the granular endoplasmic reticulum. It has been reported that in various blood vessels of smaller rodents (hamster, rat, rabbit), the immunolabelling for ET-1 observed at the ultrastructural level was predominantly associated with the cytoplasm of endothelial cells, although some labelling was also associated with the membrane of intracellular organelles, including the endoplasmic reticulum, mitochondria and cytoplasmic vesicles (Loesch *et al.* 1993, Loesch & Burnstock 1995, 1996, Shochina *et al.* 1997, Saitongdee *et al.* 1999). In the cerebrovascular endothelium of rodents, e.g. the rat or rabbit basilar artery and posterior communicating arteries, immunoreactivity for ET-1 was located in the cytoplasm throughout the cell (Loesch *et al.* 1993, Shochina *et al.* 1997). A similar ultrastructural distribution of immunoreactive ET-1 was also reported in the human middle cerebral artery free of cerebrovascular disease (Gorelova *et al.* 1996). In contrast, it has been reported that in dermal microvessels in patients with systemic sclerosis, immunoreactivity for ET-1 was highly linked with ribosomes and granular endoplasmic reticulum of endothelial cells (Tabata *et al.* 1997). This comparison of the ribosomal localisation of ET-1 in capybara and patients with systemic sclerosis shows that a similar distribution of ET-1 immunoreactivity can be observed in endothelial cells in different circumstances and vessels.

The cytoplasmic (cytosolic) immunoreactivity for ET-1 in endothelial cells is mainly linked with the constitutive release of this peptide and regulation at the transcription or translation level (Yanagisawa *et al.* 1988). The present results strongly suggest that the synthesis of ET-1 in cerebrovascular endothelial cells of the basilar artery of capybara takes place on the endoplasmic reticulum and that there is storage of the

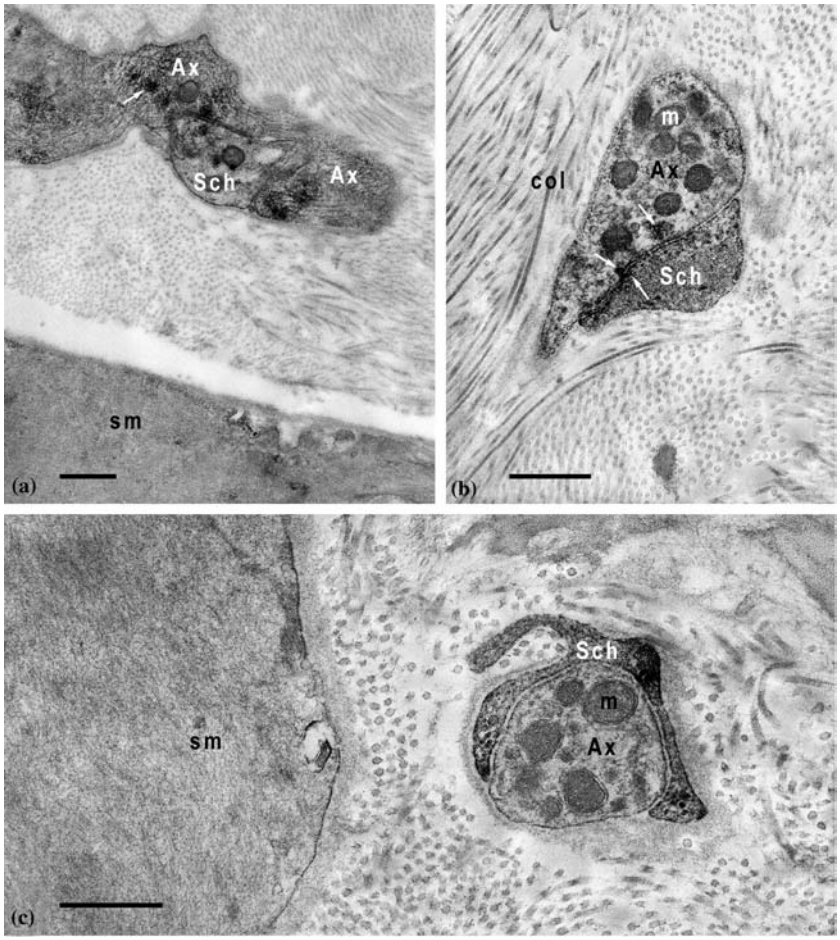

Figure 5. (a–c) Electron microscopy of perivascular nerves of capybara basilar artery immunolabelled (black precipitate) for ET_A (a) and ET_B (b, c) receptors. (a) Small nerve bundle close to the smooth muscle (sm) consisting of axon (Ax) and Schwann cell profiles (Sch) displaing immunoreactivity for ET_A; some labelling is associated with the granular vesicles (arrow). (b) Note that immunoreactivity for ET_B receptor is located in an axon varicosity and Schwann cell; clusters of immunoprecipitate (arrows) dominate in the contact regions of the cells. sv-synaptic vesicles, m-mitochondria. (c) Note that the Schwann cell displaying immunoreactivity for ET_B receptors is embracing an axon varicosity, which is immunonegative. [(a) is from a 12-month-old male; (b, c) are from 12-month-old females]. Bars: (a, b) = 0.5 μm.

peptide in the cytoplasm. Thus the endothelial cells examined appear to express various stages of synthesis and/or storage of ET-1. These results also imply that such cells were involved in the regulatory processes of cerebral blood flow. In mammals, the large cerebral arteries, including the basilar artery, are usually the major contributors to the blood flow in the mammalian brain (Bevan *et al*. 1998). This may be of particular importance following regression of the internal carotid artery in mature capybara (Reckziegel *et al*. 2001).

ET_A and ET_B receptors

The disclosure of a similar distribution of both ET_A and ET_B receptors in the capybara was unexpected. This distribution involved the endothelium, vascular smooth muscle cells, perivascular nerves (axons and Schwann cells) and fibroblasts. Usually, the ET_A receptor subtype predominates on the vascular smooth muscle and mediates vasoconstriction, whilst the ET_B receptor subtype evokes vasorelaxation when present on the endothelium (through induction of NO formation); however, vasoconstriction may also occur via ET_B receptors present on vascular smooth muscle (Arai *et al*. 1990; Masaki *et al*. 1991). Interestingly, both ET_A and ET_B receptors located on vascular smooth muscle cells of the tunica media may mediate contraction (see Vanhoutte 2000).

In human blood vessels, for example, both ET_A and ET_B receptors have been shown to be involved in ET-1-mediated contraction (Seo *et al*. 1994). The distribution and density of ET_A and ET_B receptors on vascular smooth muscle cells of the tunica media may vary from vessel to vessel as well as in physiological or pathophysiological conditions. In porcine saphenous vein to carotid artery grafts, for instance, a higher density of ET_A compared to ET_B receptors was noted in the tunica media (Dashwood *et al*. 1998). Studies of the expression of ET_A and ET_B receptor mRNA in

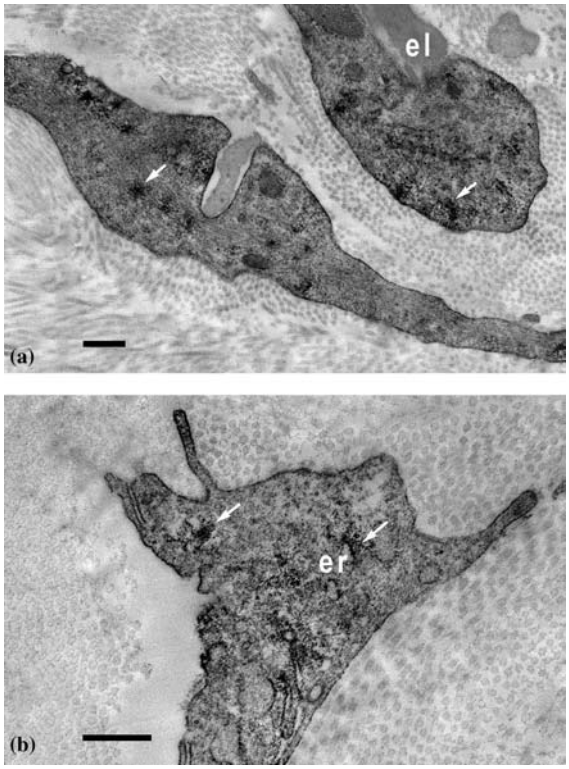

Figure 6. (a, b) Electron microscopy of adventitial fibroblasts of capybara basilar artery immunolabelled (black precipitate) for ET_A (a) and ET_B (b) receptors. Note that the immunoreactivity for ET_A and ET_B receptors appears rather patchy (arrows) in the cytoplasm; in (b) immunoreactivity is also associated with the granular endoplasmic reticulum (er). el-elastin. [(a, b) are from 12-month-old females]. Bars: 0.5 μm.

human cerebral arteries detected both types of ET receptor in endothelial cells, and an increase in the expression of ET_B receptors was observed in patients with cerebrovascular disease (Hansen-Schwartz *et al.* 2002). In the present study both receptors have been detected in both endothelial and vascular smooth muscle cells of the BA, although at rather a discrete cytoplasmic location, as revealed with electron microscopy. Light microscopic differences observed, e.g., as to the intensity of immunoreactivity for ET_A or ET_B receptors in vascular smooth muscle cells, were not obvious when examined at the ultrastructural level. It is not known at this stage which of the receptors predominates in the endothelium and vascular smooth muscle of capybara basilar artery. More systematic (quantitative) studies of capybara basilar artery are required to address this issue.

In human cerebral arteries, the contractile ET_A and dilatory ET_B receptors and their mRNAs have been reported; the mRNAs were detected both in the presence and absence of the endothelium (Nilsson *et al.* 1997). Furthermore, it has been suggested that the application of ET_A receptor antagonists may provide an additional dilatory benefit in human cerebrovascular disorders (e.g., delayed vasospasm connected with subarachnoid haemorrhage and ischaemic stroke) associated with raised ET-1 levels (Pierre & Davenport 1999). This is in concert with the view that ET-1 plays an important role in the blood-induced prolonged cerebral vasoconstriction and altered vasoreactivity that follows cerebral haemorrhage via stimulation of ET_A receptors (Yakubu & Leffler 1996).

The present study indicates that in capybara basilar artery, the ET_A and ET_B receptors are also associated with the perivascular axons, including axon varicosities and Schwann cells. This suggests a relationship exists between ET-1 and perivascular nerves as well as Schwann cells. The existence of such a relationship has previously been demonstrated e.g., in studies on the peripheral nervous system and its development (Brennan *et al.* 2000, Pomonis *et al.* 2001, Mirsky *et al.* 2002). Furthermore the expression of binding sites for ET-1 has been demonstrated on perivascular nerves of various human blood vessels (Dashwood *et al.* 1996, 1998, 2000, Dashwood & Thomas 1997). In the present study, no ET-1-positive perivascular nerves and Schwann cells were observed. The presence of ET-1-positive cerebrovascular nerves, however, has previously been reported in rat basilar artery and middle cerebral artery in man (Loesch *et al.* 1998, Milner *et al.* 2000a, Loesch & Burnstock 2002, Mickey *et al.* 2002); in addition, ET-1-positive Schwann cells have been observed in human middle cerebral artery in a case of multiple system atrophy with autonomic failure (Loesch *et al.* 2004). The possibility cannot be excluded that ET-1-positive cerebrovascular nerves exist in capybara cerebral vessels but that we failed to detect them. It is also possible that there is a change in the innervation to the basilar artery in capybara due to the regression of the internal carotid artery at the animal's maturation (Reckziegel *et al.* 2001); this is at a period between 6 and 12 months of life. In fact the details of the innervation and the type of autonomic ganglia projecting to the cerebral vessels of capybara are unknown. This study shows that at least some perivascular nerves display immunoreactivity for ET_A and ET_B receptors. Furthermore, the ET_A receptors seem localised with granular synaptic vesicles in varicosities suggesting an involvement in neurotransmission. Indeed, studies of peripheral nerves indicate that at least ET_A receptors can be localised to peptidergic and non-peptidergic sensory nerves and their axons (Pomonis *et al.* 2001).

The present study also demonstrated immunoreactivity of adventitial fibroblasts for ET-1 and ET receptors suggesting an important role of these cells in the ET-1-related mechanisms in the basilar artery of capybara. Indeed, this observation supports the data showing that adventitial fibroblasts are able to generate ET-1 under a variety of circumstances e.g., in mouse aorta and mesenteric arteries in response to oxidative

stress or angiotensin II stimulation (Wang & Wang 2003, Wang & Zhu 2003). ET-1 may modulate remodelling of extracellular matrix by promoting fibroblast synthesis of collagen types I and III, a process that depends upon both ET_A and ET_B receptors (Shi *et al.* 2001). This may have particular significance in relation to the basilar artery of capybara, as this artery is likely to undergo various remodelling processes due to the regression of the internal carotid artery (Reckziegel *et al.* 2001, Islam *et al.* 2004). Our preliminary unpublished observations suggest that in 12-month-old animals the basilar artery may double its diameter (from ~1.2 to 2.6 mm), which is probably associated with artery overload following regression of the internal carotid artery. Whether this substantial anatomical and physiological change is interrelated with the expression of ET-1 and ET receptors is unknown at this stage.

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