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Endothelin ET_A receptor expression in human cerebrovascular smooth muscle cells

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1 Endothelin (ET) has been implicated in cerebrovasospasm for example, following subarachnoid haemorrhage, and blocking the interaction of ET with its receptors on cerebral vessels, may be of therapeutic benefit. The aim of our study was to characterize endothelin receptor sub-types on medial smooth muscle cells of human cerebral vessels. Cultures of vascular smooth muscle cells were explanted from human cerebral resistance vessels and characterized as human brain smooth muscle cells (HBSMCs).

2 Over a 48 h incubation period, HBSMC cultures secreted comparable levels of immunoreactive (IR) big endothelin-1 (big ET-1) and IR endothelin (ET): 12.7 ± 10.3 and 8.3 ± 5.6 pmol/10^6 cells, respectively (mean $\pm$ s.e.mean from three different individuals), into the culture medium.

3 Total RNA was extracted from cultures of human brain smooth muscle cells. Reverse-transcriptase polymerase chain reaction (RT-PCR) assays and subsequent product separation by agarose gel electrophoresis revealed single bands corresponding to the expected product sizes encoding cDNA for ET_A (299 base pairs) and ET_B (428 base pairs) ($n=3$ different cultures).

4 Autoradiography demonstrated the presence of specific binding sites for [^{125}I]-ET-1 which labels all ET receptors, and [^{125}I]-PD151242, an ET_A subtype-selective antagonist which exclusively labels ET_A receptors, but no specific binding was detected using ET_B subtype-selective [^{125}I]-BQ3020 ($n=3$ different cultures, in duplicate).

5 In saturation binding assays, [^{125}I]-ET-1 bound with high affinity: $K_D = 0.8 \pm 0.1$ nM and $B_{max} = 690 \pm 108$ fmol mg^{-1}. A one-site fit was preferred and Hill slopes were close to unity over the concentration range (10^{-12} to 10^{-8} M). [^{125}I]-PD151242 also bound with similar affinity: $K_D = 0.4 \pm 0.1$ nM and $B_{max} = 388 \pm 68$ fmol mg^{-1} (mean $\pm$ s.e.mean, $n=3$ different cultures). Again, a one-site fit was preferred and Hill slopes were close to unity over the concentration range. Unlabelled PD151242 competed for the binding of [^{125}I]-ET-1 monophasically and analysis of the competition curves indicated that a one-site fit was preferred over a two-site model, implying that the cultures express mainly ET_A receptors.

6 Although messenger RNA encoding both ET_A and ET_B receptors was detected, autoradiographical analysis, as well as binding studies indicate that human cultured brain smooth muscle cells express only ET_A receptor protein. Antagonism of this sub-type may be necessary to block the actions of ET-1 in the human cerebral resistance vessels in the vasospasm observed subsequent to subarachnoid haemorrhage.

Keywords: Endothelin; endothelin receptors; PD151242; BQ3020; cerebral resistance vessels

Introduction

The endothelins (ETs) are potent constrictors of peripheral blood vessels which are thought to act through distinct ET_A and ET_B receptor sub-types (Arai *et al.*, 1990; Sakurai *et al.*, 1990). Endothelin also produces protracted vasoconstriction *in vivo* animal models (Kobayashi *et al.*, 1990) and *in vitro* strips of human cerebral arteries obtained post-mortem (Papadopoulos *et al.*, 1990). Thus, it has been hypothesized that endothelin may play a role in the pathogenesis of cerebrovascular diseases such as stroke, or cerebral vasospasm following subarachnoid haemorrhage. Evidence of elevated immunoreactive (IR) ET levels in plasma and CSF from human, as well as from animal models has been documented, which may support this hypothesis. In human subjects, elevated levels of endothelin-like immunoreactivity in plasma (Masaoka *et al.*, 1989; Levesque *et al.*, 1990) and cerebrospinal fluid (Suzuki *et al.*, 1990) were demonstrated in patients with subarachnoid haemorrhage, but once established they appeared to be associated with a poor prognosis since the elevation of IR ET or big ET-1 in CSF coincided with clinically documented signs of cerebral vasospasm (Suzuki *et al.*, 1992; Ehrenreich *et al.*, 1992; Seifert *et al.*, 1995). In animal models, a role for ET-1 has been implicated in spontaneously hypertensive stroke-prone rats (Yamashita *et al.*, 1993). Intra-arterial injection of big ET-1 caused the onset of SAH in a rabbit model of middle cerebral artery occlusion (Huneidi *et al.*, 1991), whilst models of cerebral ischaemia in rabbit and rat demonstrated significantly increased IR ET in the damaged brain and neural tissue (Viossat *et al.*, 1993; Bian *et al.*, 1994; Yamashita *et al.*, 1994). It is possible, that as a potent vasoconstrictor with protracted effects, endothelin may have a causative role in these pathological conditions. Furthermore, there is evidence that the pathological effects of ET may be mediated through interaction with ET_A receptors. Intracisternal administration of the ET_A-selective antagonist, FR139317, in dogs (Nirei *et al.*, 1993) and either the ET_A-selective antagonist BQ123, or the orally active non-selective, non-peptide antagonist, Ro462005, in the rat (Clozel & Watanabe, 1993; Clozel *et al.*, 1993) prevented the development of cerebral vasospasm after subarachnoid haemorrhage. Thus, blocking the vasoconstrictor effects of ET-1 by employing ET receptor antagonists on cerebral vessels, may be a potential therapeutic target.

In human peripheral blood vessels, we have previously

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shown that the smooth muscle layer predominantly expresses ET_A receptors, with a small population of ET_B receptors (<15%) detected (Davenport & Maguire, 1994; Davenport *et al.*, 1995). Our aim was to determine whether a similar pattern occurs in smooth muscle of the cerebral vasculature. As an initial step, we characterized endothelin receptors on smooth muscle cells cultured from resistance vessels in the brain cortex. These vessels were chosen as they are routinely available and have the advantage of avoiding any post-mortem changes. Human brain smooth muscle cells were cultured in order to obtain sufficient quantities for experimentation. We tested whether human brain smooth muscle cells secreted IR ET and/or big ET-1 since we have previously shown that human vascular smooth cells cultured from peripheral vessels secrete both peptides (Yu & Davenport, 1995). We employed RT-PCR to determine whether these cells express mRNA for ET_A and ET_B receptors, and performed receptor autoradiography, as well as binding assays to identify the ET receptor subtypes present on human cerebral resistance vessels.

Methods

Tissue collection

Frontal cortices were obtained following lobectomy for removal of deep-seated malignant gliomas from seven male and female patients. Vessels were removed from the superficial regions of the lobectomy containing histologically normal cortical tissue, and cerebral vessels (diameter <300 μm) were dissected. Whilst we cannot exclude the possibility that some of the vessel was removed from the tumour itself, by imaging criteria prior to surgery, there was no evidence for leakage from these vessels in the areas of normal brain tissue. The age range was between 47 and 71 years, and all patients were premedicated with dexamethasone prior to surgery. Other medications included analgesics, antibiotics, anticoagulants, benzodiazipine, and histamine H_2-antagonists. Procedures were performed with approval from the Local Research Ethics Committee.

Cell culture

Smooth muscle cell cultures were obtained as previously described (Yu & Davenport, 1995). The endothelium was removed from vessels and they were cut into approximately 0.2 cm^2 portions for explantation of smooth muscle cells. Separate cultures were grown and maintained from vessels obtained from each patient and used separately in experiments, without pooling of cultures. HBSMC cultures were maintained in complete medium (Medium 199 (Gibco BRL, Paisley, Scotland) supplemented with 50 iu ml^{-1} penicillin, 50 $\mu g\ ml^{-1}$ streptomycin, 2.5 $\mu g\ ml^{-1}$ amphotericin B) containing 15% heat-treated foetal calf serum (Seralab, Sussex, U.K.). Culture medium was changed two to three times per week. All cell growth occurred in a humidified incubator at 37°C in which the atmosphere was equilibrated with 5% CO_2.

Characterization of VSMC cultures

For immunocytochemical studies, HBSMCs were stained for von Willebrand factor (vWF) (Dako, High Wycombe, Buckinghamshire) and for smooth muscle α-actin (Sigma). Secondary antibodies conjugated to fluorescein isothiocyanate (FITC) were employed to visualize the staining patterns. Propidium iodide was used to counterstain nuclei. IR vWF was distributed on endothelial cells in a typical punctate pattern, but endothelial cell did not stain for α-actin. Conversely, IR vWF was not detectable on HBSMCs while α-actin was distributed as a filamentous network.

Molecular biology

HBSMCs were grown to confluence in gelatin-coated 12-well plates (Flow Laboratories Inc., McLean, Virgina, U.S.A.). Total RNA was isolated by a guanidinium thiocyanate/isopropanol method with minor modifications (Chomczynski & Sacchi, 1987). Reverse transcription coupled with polymerase chain reaction was carried out as previously described (O'Reilly *et al.*, 1992; Davenport *et al.*, 1993). Between 2–10 μg total RNA were annealed to oligo(dT) primer (final concentration, 100 $\mu g\ ml^{-1}$) by heating to 68°C for 5 min then chilling on ice for 2 min. To this were added deoxy-NTPs (final concentration, 1 mM), reverse transcriptase buffer (final concentration, 50 mM Tris-HCl, pH 8.3 at 42°C; 40 mM KCl; 6 mM $MgCl_2$; 0.4 mM dithiothreitol), 0.5 μl (20 u) RNAsin, and 0.5 μl (10 u) avian myoblastosis virus reverse transcriptase enzyme in a final volume of 30 μl. First strand cDNA synthesis was carried out at 42°C for 1 h. The reverse transcriptase was inactivated by heating at 80°C for 10 min before using the cDNA. PCR amplification was performed using nested oligonucleotide primers designed from published nucleotide sequences: (A) ET_A, ET_B forward TATCACAGAAACTAAAGTGCTATG (base pairs 379–396) (B) ET_A, ET_B reverse CAAGCAAGCAAGCAACGTAAGAGCA (base pairs 1,214–1,198) (C) ET_A forward CCTTTTGATCACAATGACTTT (base pairs 439–459) (D) ET_A reverse TTTGATGTGGCATTGAGCATACAG (base 737–714) (E) ET_B forward ACTGGCCATTTGGAGCTGAGAT (base pairs 497–519) (F) ET_B reverse CTGCATGCCACTTTTCTTTCTCAA (base pairs 924–901). A 50 μl reaction mix, overlaid with 50 μl light mineral oil, contained 1 μl cDNA, 5 μl reaction buffer (100 mM Tris-HCl, pH 8.3, at 25°C, 500 mM KCl, and 15 mM $MgCl_2$), 5 μl deoxyNTPs (2 mM), 5 μl of each oligonucleotide primer (10 μM), and 2.5 u Taq polymerase. Nested PCR was carried out by taking 1 μl reaction mix from the first round of amplification and performing a second round of amplification in a fresh 50 μl reaction using the internal primer pair. The amplification profile for the primers was as follows: Primers A, B: (95°C, 0.5 min; 40°C, 0.5 min; 72°C, 1.0 min) × 20 cycles. The first cycle for each reaction was preceded by denaturation at 95°C for 1.0 min, and the final cycle was followed by strand extension at 72°C for 3.0 min. The PCR products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining.

Radioimmunoassay

To determine secreted levels of big ET-1 and mature ET, supernatants from triplicate wells of three different HBSMC cultures were collected, and the cells were washed twice with PBS. Cells were then trypsinized to obtain estimates of cell number using a haemocytometer. The culture supernatants were directly radioimmunoassayed for mature ET and big ET-1 as previously described (Plumpton *et al.*, 1992). Briefly, the mature ET antisera were raised against the C-terminus of ET-1 [15-21] and showed 100% cross-reactivity with ET-2 and ET-3. Antisera against big ET-1 was raised against the C terminus [31-38] and showed <0.007% cross-reactivity with the mature peptides (ET-1, ET-1 and ET-1), big ET-2 and big ET-3. The sensitivity of detection of both assays was 0.4 fmol/tube. The inter- and intra-assay variations were <13% in all cases. [^{125}I]ET-1 (~2000 Ci $mmol^{-1}$) and [^{125}I]big ET-1 (~2000 Ci $mmol^{-1}$) were obtained from Amersham International, Buckinghamshire.

Autoradiography

ET_A and ET_B receptors were visualized by published autoradiographical techniques (Molenaar *et al.*, 1992; Maguire *et al.*, 1994). HBSMCs from three different vessels were grown to confluence on glass coverslips, in triplicate, and preincubated for 15 min at room temperature (23°C) in incubation buffer (50 mM HEPES containing 5 mM $MgCl_2$,

bovine serum albumin, 0.03% w/v). Cells were then incubated for 1 h in the same buffer containing either 0.1 nM [^{125}I]-ET-1 (~2000 Ci mmol^{-1}) to detect all ET receptors, or the ET_A-selective [^{125}I]-PD151242 (N-[-hexahydro-1-azepinyl(carbonyl)L-Leu(1-Me)D-Trp-D-tyr) (~2000 Ci mmol^{-1}) or the ET_B-selective [^{125}I]-BQ3020 [Ala^{11,15}]Ac-ET-$1_{(6-21)}$ (~2000 Ci mmol^{-1}). Non-specific binding was determined by incubating coverslips in the presence of 1 μM of the corresponding unlabelled peptide. At the end of the incubation period, cells were rinsed in three successive 5 min washes of ice cold Tris buffer (pH 7.4) and dried. Sections were exposed for five days to radiation sensitive film, Hyperfilm βmax (Amersham International, Buckinghamshire).

Saturation and competition binding assays

HBSMCs were plated onto a collagen-coated Costar 96-well strip plate with low evaporation lid (Cambridge, MA, U.S.A.) and grown to confluence. A Titertek Microplate Washer (ICN/Flow, High Wycombe, Bucks) was used to wash the cells. For saturation binding experiments, HBSMCs were incubated with increasing concentration of [^{125}I]-ET-1 or [^{125}I]-PD151242 (Amersham International, Buckinghamshire, U.K.) (concentration range: 10^{-12} M – 10^{-8} M). For competition experiments HBSMCs were incubated with 100 pM [^{125}I]-ET-1 and increasing concentrations of unlabelled PD151242 (concentration range: 2×10^{-11} M – 10^{-4} M). Non-specific binding was determined by the inclusion of 1 μM unlabelled ET-1 as previously described (Davenport *et al.*, 1993; 1994; Peter & Davenport, 1995). Dissociation constants and receptor density were estimated using EBDA (McPherson, 1983) and an iterative non-linear curve-fitting programme LIGAND (Munson & Rodbard, 1980).

Protein determinations

Protein concentrations of washed cells were estimated after solubilization (0.5 M NaOH and 1% sodium dodecyl sulphate) for 5 min at room temperature. The Bio-Rad Detergent Compatible 96-well microtiter plate method (BioRad Laboratories, Hertfordshire, U.K.) was used with bovine serum albumin as a reference standard.

Statistical analysis

K_D and B_{max} values were analysed by a Mann-Whitney U-test with significance set at $P<0.05$. Results are given as mean ± s.e.mean.

Results

HBSMC cultures were characterized as smooth muscle by the presence of smooth muscle α-actin filaments and the concomitant absence of von Willebrand factor, an established marker of endothelial cells. Radioimmunoassays demonstrated that over a 48 h incubation period, HBSMCs secrete comparable levels of both IR big ET-1 and IR ET: 12.7 ± 10.3 and 8.3 ± 5.6 pM/10^6 cells, respectively (mean ± s.e.mean, $n=3$ different cultures from 3 separate vessels). In cultures of HBSMCS, reverse-transcriptase polymerase chain reaction assays and subsequent product separation by agarose gel electrophoresis revealed single bands corresponding to the expected product sizes encoding cDNA for ET_A (299 base pairs) and ET_B (428 base pairs) ($n=3$ different cultures). Figure 1 shows an example of cDNA for ET_A and ET_B receptors from a culture of HBSMCs, present at the expected position as compared to the positive control of human placental cDNA which express mRNA for both ET receptor subtypes.

Using separate cultures from three individuals, receptor autoradiography illustrated the presence of specific binding sites for [^{125}I]ET-1 and ET_A selective [^{125}I]-PD151242 (Figure 2a, b, c and d), which label all ET receptors, and ET_A receptors respectively, but no specific binding was detected using ET_B subtype-selective [^{125}I]-BQ3020 (Figure 2e, and f). An example of the autoradiograms is shown in Figure 2.

Saturation binding assays revealed similar K_D values of 0.8 ± 0.1 nM and 0.4 ± 0.1 nM (mean ± s.e.mean, $n=3$) using [^{125}I]-ET-1 and [^{125}I]-PD151242, respectively. Although they displayed greater variability, B_{max} values were observed to be 690 ± 108 fmol mg^{-1} using [^{125}I]-ET-1, whilst ET_A selective [^{125}I]-PD151242 yielded a similar value of 388 ± 68 fmol mg^{-1} (mean ± s.e.mean, $n=3$). These receptor densities were not statistically different ($P>0.05$) (Table 1). With either radioligand, a one-site fit was preferred to a two-site model, and Hill slopes were close to unity over the concentration-range, indicating the presence of a single population of receptors. In competition binding assays, the ET_A subtype-selective antagonist, PD151242, competed for the binding of [^{125}I]-ET-1 monophasically and analysis of the competition curves indicated that a one-site fit was preferred over a two-site model ($n=2$) (Figure 3).

Discussion

In the present study, we have shown that human cultured cerebral vascular smooth muscle cells secrete both IR ET and big ET-1. We have previously demonstrated the secretion of ET-1, ET-3 and big ET-1 from smooth muscle cells cultured from human peripheral vessels such as aorta, coronary artery, mammary artery, saphenous vein and umbilical vein (Yu & Davenport, 1995). Peripheral vessels express mRNA for ET_A and ET_B receptors (Davenport *et al.*, 1993); however, competition binding assays indicated the predominance of ET_A receptors with ET_B receptors constituting less than 15% of the total ET receptor population in coronary artery, pulmonary artery, renal artery, aorta and saphenous vein (Davenport *et al.*, 1993; Maguire *et al.*, 1994). Similarly, in the present study, we were able to detect single bands for cDNA encoding either ET_A or ET_B receptors in cultured HBSMCs. Recent evidence demonstrates that two distinct human ET_B receptors can be generated by alternative splicing from a single gene (Shyamala

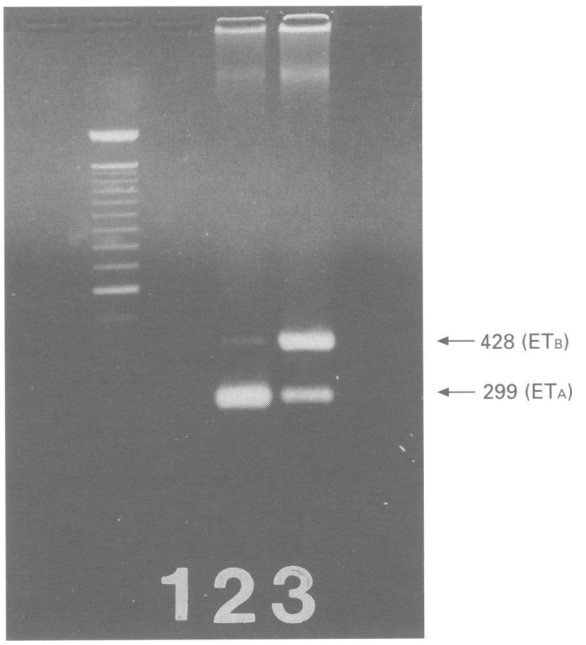

Figure 1 Agarose gel showing products of reverse transcriptase-polymerase chain reaction assay for cDNA from cultured HBSMCs amplified with ET_A and ET_B specific primers. In lane 1, the DNA template has been omitted as a negative control. Lanes 2 and 3 show the presence of specific bands corresponding to the size predicted for ET_A (299 base pairs) and ET_B (428 base pairs) for HBSMCs and human placenta as a positive control, respectively.

et al., 1994). However, our primers span this splice region, and the lack of additional bands in our assay do not suggest the presence of other ET_B subtypes. Despite the expression of mRNA for ET_A and ET_B receptors, HBSMCs express predominantly ET_A subtype receptors, and they display similar affinity to ET_A receptors present in the peripheral vasculature. Saturation binding assays on HBSMCs yielded K_D values of 0.8 ± 0.1 nM for [^{125}I]ET-1 and 0.4 ± 0.1 nM for [^{125}I]-PD151242, similar to K_D values observed for [^{125}I]ET-1 binding in pulmonary artery, 0.85 ± 0.25 nM and in aorta, 0.51 ± 0.02 nM (Davenport *et al.*, 1995). In competition binding studies, the affinity of PD151242 for the HBSMCs was in the nanomolar range, which parallels previous observations in other human tissue such as cardiac arteries, kidney and left ventricle of the heart (Davenport *et al.*, 1994; Peter & Davenport, 1995). Although HBSMCs express mRNA for ET_A and ET_B receptors, in accordance with previous observations for peripheral vessels, our present study demonstrated the predominant expression of ET_A receptors on HBSMCs.

The precise physiological or pathophysiological role of ETs in human cerebral vessels remains unclear. Similar to previous observations for endothelial cells from peripheral vessels, human cerebromicrovascular endothelium has been shown to constitutively produce ET-1 (Yoshimoto *et al.*, 1990; Bacic *et al.*, 1992), which can be upregulated by arginine vasopressin or angiotensin II (Spatz *et al.*, 1994). Endothelin production from bovine endothelial cells (Ohlstein & Storer, 1992; Kasuya *et al.*, 1993), as well as from rat SMCs (Kasuya *et al.*, 1993), has also been demonstrated to be upregulated by oxyhaemoglobin, which is likely to be one of the principal pathogenic agents responsible for cerebral vasospasm following subarachnoid haemorrhage (Macdonald & Weir, 1991). In addition, endothelins have been shown to increase permeability of human cultured cerebromicrovascular endothelium (Stanimirovic *et*

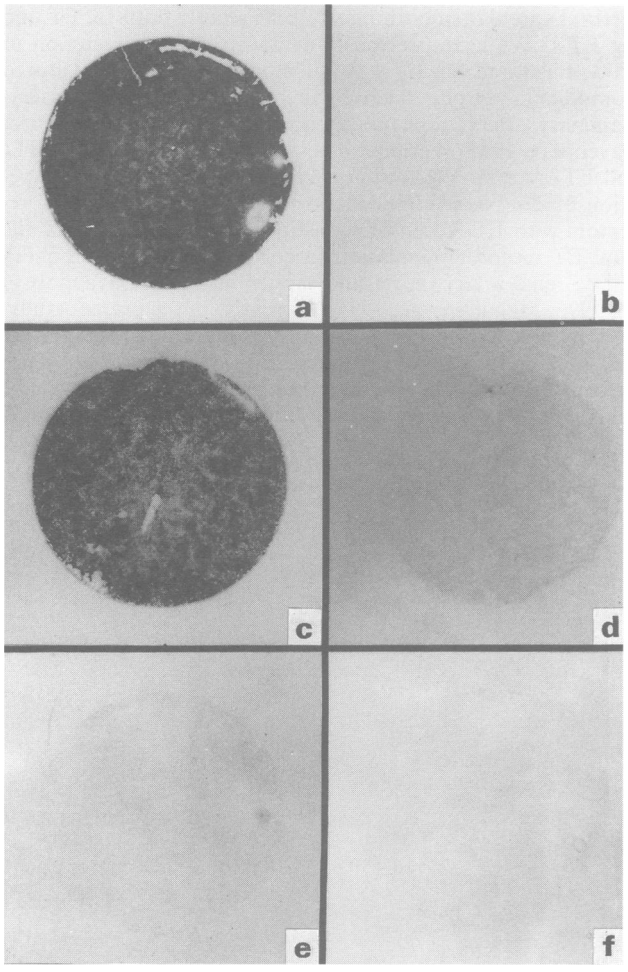

Figure 2 Photomicrograph of typical autoradiograms performed on HBSMCs incubated with 0.1 nM [^{125}I]-ET-1 to detect all ET receptors, or the ET_A-selective, [^{125}I]-PD 151242 or the ET_B-selective [^{125}I]-BQ3020. Non-specific binding was determined by incubating adjacent coverslips in the presence of 1 μM of the corresponding unlabelled peptide. Panels (a) and (b) show total [^{125}I]-ET-1 total binding and non-specific binding, respectively. Panels (c) and (d) represent cells labelled with ET_A-selective [^{125}I]-PD151242 to show location of ET_A receptors, and its corresponding non-specific binding. Panels (e) and (f) illustrate that ET_B-selective [^{125}I]-BQ3020 total binding and non-specific binding are nearly indistinguishable. The ET receptor population expressed on cultured HBSMCs is mainly ET_A, with undetectable amounts of ET_B.

Table 1 Binding parameters for human cultured cerebral smooth muscle cells, obtained from saturation binding assays using [^{125}I]ET-1 or [^{125}I]-PD151242

Compound	n	K_D [nM]	B_{max} (fmol mg^{-1})
[^{125}I]-ET-1	3	0.8 ± 0.1	690 ± 108
[^{125}I]-PD151242	3	0.4 ± 0.1	388 ± 68

Each value represents the mean ± s.e.mean of 3 different cultures. The concentration-range of labelled ligand was 10^{-12} to 10^{-8} M. Saturation binding assays using [^{125}I]-ET-1 and [^{125}I]-PD151242 yielded binding parameters which were not statistically different. Since PD151242 is an ET_A-selective compound, this similarity indicates that the total ET receptor population expressed on cultured human brain smooth muscle cells is mainly the ET_A subtype. Competition binding using PD151242 against [^{125}I]-ET-1 confirmed the presence of a single population of receptors.

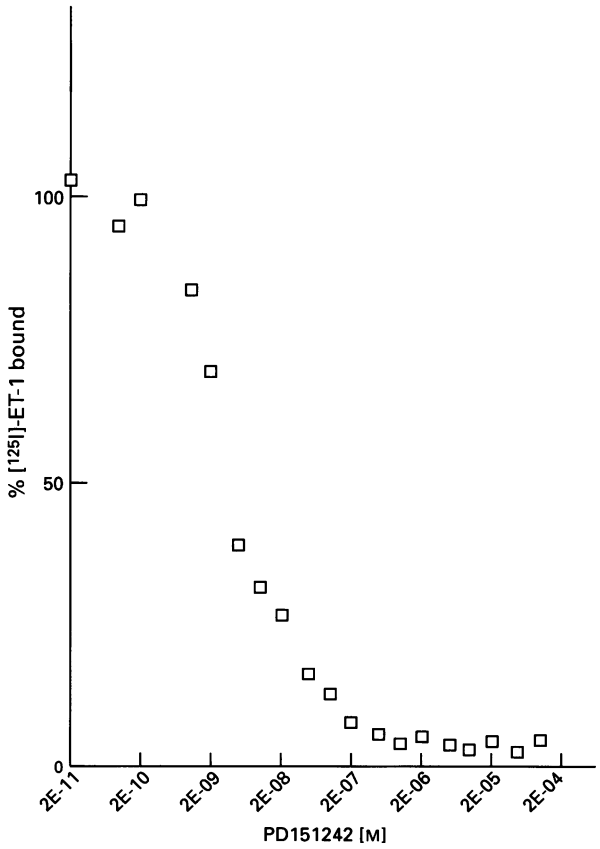

Figure 3 Competitive binding of 100 pM [^{125}I]-ET-1 to HBSMCs by unlabelled PD151242 (concentration range: 2×10^{-11} to 10^{-4} M). The monophasic inhibition curve shows that PD151242 competes at concentrations greater than 0.1 nM for almost 100% of the specific [^{125}I]-ET-1 binding sites. Points represent the average of two separate experiments on two different cultures of HBSMCs.

al., 1993), which may contribute to alterations of the blood-brain barrier. These findings suggest that excessive release of ET-1 may contribute to the alterations of blood-brain barrier permeability observed during the course of cerebrovascular diseases such as stroke, hypertension and cerebrovasospasm following subarachnoid haemorrhage. Elevated levels of IR ET detected in plasma (Masaoka *et al.*, 1989; Suzuki *et al.*, 1992) and CSF (Suzuki *et al.*, 1990; 1992; Ehrenreich *et al.*, 1992) of SAH patients, may be a reflection of this excessive release of ET from the endothelium of cerebral vessels, or their medial SMCs. The secretion of IR ET from HBSMCs may represent the induction of ET from phenotypically modulated or 'diseased' VSMCs, which may participate in the pathogenesis of cerebrovascular pathologies. Accordingly, normal astrocytes do not show IR ET, but expression has been noted in cases of infarcts, lacunes, hereditary multi-infarct disease, Binswanger's encephalopathy and Alzheimer's disease (Zhang *et al.*, 1994), indicating that in a pathological setting, the induction of ET may also occur in cell types of the central nervous system. Cultured VSMCs from peripheral vessels are phenotypically modulated and display cytoskeletal features similar to foetal or pathological VSMCs *in vivo*. Likewise, our cultured HBSMCs may reflect phenotypically modulated, pathological VSMCs in diseased cerebral vessels, and as with their peripheral counterparts, the observation that HBSMCs are a potential source of endothelin secretion may be indicative of a role for ET in the development of cerebral ischaemic injury and in the pathogenesis of cerebral disorders such as stroke or cerebral vasospasm following subarachnoid haemorrhage.

It is feasible that ET secretion in cerebral vessels is regulated by various factors, as is the situation for peripheral vessels, and the elevated levels of ET noted in cerebrovascular pathologies, may be a passive response to the withdrawal of an inhibitory factor. In porcine aorta, ET secretion has been shown to be downregulated by vasodilators NO and PGI_2 (Boulanger & Lüscher, 1990), and similarly, Edvinsson *et al.* (1994) reported that the level of calcitonin gene-related peptide (CGRP), a potent vasodilator, was significantly lower in arteries removed from SAH patients. The release of CGRP from sensory nerve fibres indirectly mediates the activation of an endothelium-derived relaxing factor (Wei *et al.*, 1992) which does not appear to be nitric oxide (Rosenblum, 1992). Thus, it is possible that the elevated plasma and CSF levels of ET observed in these patients is related to a diminished negative feedback mechanism within the CNS.

Given the conceivable role of ET in cerebral disease, endothelin receptor antagonists may yield potentially therapeutic benefits. As in the peripheral vasculature, we have shown the predominance of ET_A receptors on HBSMCs, and there is functional evidence that cerebral vessels from rat neocortex (Sagher *et al.*, 1994) and human cerebral, meningeal and temporal arteries (Adner *et al.*, 1994), express mainly ET_A receptors. However, unlike peripheral vascular endothelium, human brain endothelial cells also appear to possess mostly ET_A receptors (Stanimirovic *et al.*, 1994). Furthermore, there is mounting evidence from various animal model systems, that the interaction of ET with ET_A receptors may be involved in the development of cerebrovascular pathologies. Itoh *et al.* (1993; 1994) reported that continuous, intrathecal administration of ET_A antagonists BQ485 or BQ123, prevents narrowing of canine basilar artery in cerebrovasospasm, with an elevation of mRNA for ET_A receptors on day 7 after SAH. Intracisternal administration of the ET_A-selective antagonist, FR139317 in dogs (Nirei *et al.*, 1993), and ET_A-selective antagonist, BQ123 in rat (Clozel & Watanabe, 1993) prevented the development of cerebral vasospasm after subarachnoid haemorrhage. The evidence suggests a role for endothelin, possibly through interaction with ET_A receptors expressed on cerebrovascular endothelial or smooth muscle cells, in the pathogenesis of stroke and/or cerebral vasospasm following subarachnoid haemorrhage. Thus, the cited evidence, as well as the results of our present study that human brain smooth muscle cells express mainly ET_A receptors, suggest that antagonism of this sub-type may be sufficient to block the pathological effects of excessive ET secretion. This would leave intact any beneficial endothelial ET_B-dependent, and possibly regulatory, effects such as the production of vasodilators prostacyclin or nitric oxide.

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