Review

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Endothelin systems in the brain: involvement in pathophysiological responses of damaged nerve tissues

Abstract: In addition to their potent vasoconstriction effects, endothelins (ETs) show multiple actions in various tissues including the brain. The brain contains high levels of ETs, and their production is stimulated in many brain disorders. Accumulating evidence indicates that activation of brain ET receptors is involved in several pathophysiological responses in damaged brains. In this article, the roles of brain ET systems in relation to brain disorders are reviewed. In the acute phase of stroke, prolonged vasospasm of cerebral arteries and brain edema occur, both of which aggravate brain damage. Studies using ET antagonists show that activation of ET_A receptors in the brain vascular smooth muscle induces vasospasm after stroke. Brain edema is induced by increased activity of vascular permeability factors, such as vascular endothelial growth factor and matrix metalloproteinases. Activation of ET_B receptors stimulates astrocytic production of these permeability factors. Increases in reactive astrocytes are observed in neurodegenerative diseases and in the chronic phase of stroke, where they facilitate the repair of damaged nerve tissues by releasing neurotrophic factors. ETs promote the induction of reactive astrocytes through ET_B receptors. ETs also stimulate the production of astrocytic neurotrophic factors. Recent studies have shown high expression of ET_B receptors in neural progenitors. Activation of ET_B receptors in neural progenitors promotes their proliferation and migration, suggesting roles for ET_B receptors in neurogenesis. Much effort has been invested in the pursuit of novel drugs to induce protection or repair of damaged nerve tissues. From these studies, the pharmacological significance of brain ET systems as a possible target of neuroprotective drugs is anticipated.

Keywords: brain edema; endothelin; neurodegenerative disease; neuroprotection; stroke; vasospasm.

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Introduction

Since their discovery as a novel peptide family (1), the functions of endothelins (ETs) have been intensively investigated in the circulatory system, because of their potent vasoconstriction effects. However, soon after their discovery, it was shown that ET ligands and ET receptors are present in various tissues, and multiple functions for ET systems were postulated (2). Accumulating evidence indicates regulatory roles for ETs in functions other than vascular tone, such as hypertrophy, fibrosis, inflammation, and various other physiological and pathological functions. Several ET receptor agonists and antagonists have been developed (Table 1). At present, there are many selective ET agonists and antagonists, some of which are now used clinically (3, 4). Since the discovery of ETs, ET ligands and their receptors have been known to be highly expressed in the brain (5, 6). Therefore, specialized roles for ET systems in the nervous system have been postulated. In addition to roles in neurotransmission and embryonic development, investigations of brain ET systems have shown their significance in brain disorders including Alzheimer's disease (AD) and stroke (7–9). Development of effective treatments for brain dysfunctions in neurodegenerative diseases and stroke is still greatly needed. Many neuroscientists are engaged in searches for novel targets of neuroprotective drugs. In this article, recent studies examining the possible roles of brain ET systems in the pathophysiological responses of damaged brains are reviewed.

ET ligands

The ET peptide family consists of three isopeptides: ET-1, ET-2, and ET-3 (Figure 1). These endogenous ET ligands have a structural similarity; they comprise 21 amino acids with two disulfide bonds. In humans, ET-1, ET-2, and ET-3 are encoded as large precursor proteins, prepro-ETs, by distinct genes. The biological actions of ETs are

Table 1 Agonists and antagonists for ET receptors.

	Agonists	Antagonists
ET non-selective	ET-1	SB209670, bosentan, TAK-044, tezosentan
ET_A selective	Sarafotoxin 6b	BQ123, darusentan, ambrisentan, sitaxsentan, clazosentan, S-0139, SB234551, Ro-61-1790
ET_B selective	Sarafotoxin 6c, IRL-1620, BQ3020, $Ala^{1,3,11,15}$-ET-1	BQ788, IRL-2500, A-192621, RES-701-1

mediated by two types of receptors: ET_A and ET_B receptors. Because ET-1 is the most abundant isopeptide in many tissues, many investigations of ET ligands have focused on ET-1. ET-1 activates both ET_A and ET_B receptors, and induces biological effects including vasoconstriction and proliferation (2–4). ET-2 differs from ET-1 by two amino acids, but has similar receptor selectivity to ET-1. ET-2 is highly expressed in intestine, ovary, and pituitary glands (10–12). Owing to similar receptor selectivity, the biological actions of ET-2 were originally thought to overlap with ET-1. However, recent studies have shown that ET-2 has distinct actions from ET-1 (13, 14). ET-3, which differs from ET-1 by six amino acids, is abundant in the intestine, lung, and brain (2, 7). ET-3 has high affinity for ET_B receptors,

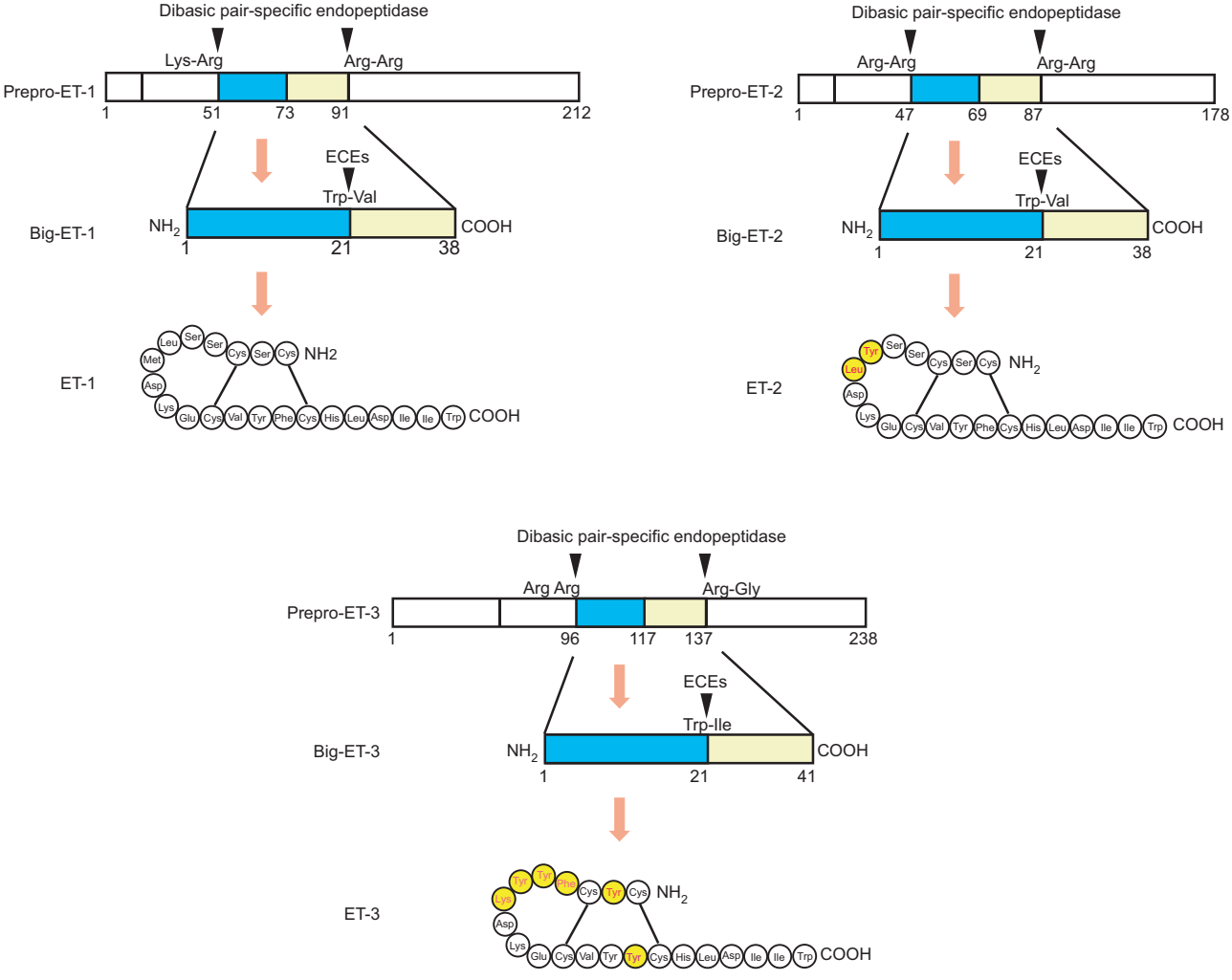

Figure 1 Biosynthesis of human ETs from prepro-ETs.
ET-1 is translated as an inactive precursor protein called prepro-ET-1. Prepro-ET-1 is cleaved by dibasic pair-specific endopeptidases and converted to big-ET-1. Specific processing of big-ET-1 by endothelin-converting enzymes (ECEs) results in production of mature ET-1. There are three distinct ET family peptides, ET-1, ET-2, and ET-3, all of which consist of 16 amino acids and two intramolecular disulfide bonds and are produced by a similar process to ET-1.

but not ET_A receptors, indicating that it is an endogenous ET_B ligand. Gene knockout of either ET-3 or ET_B receptors in mice results in a similar impairment in enteric neuron development (15, 16). These mouse phenotypes resemble Hirschsprung's disease, and in accordance with this, patients with Hirschsprung's disease have a mutated ET_B receptor gene (17–19).

As occurs for many peptide hormones, the active forms of ETs are produced by cleavage of their precursor peptides (Figure 1). For human ET-1, the 212 amino acid inactive precursor protein (prepro-ET-1) is translated from its mRNA. Prepro-ET-1 is cleaved by a dibasic pair-specific endopeptidase at Lys^{51}-Arg^{52} and Arg^{92}-Arg^{93} to produce a 38 amino acid precursor, big-ET-1. The active form of ET-1 is produced from big ET-1 after processing at Trp^{21}-Val^{22} by specific proteases called endothelin converting enzymes (ECEs). Although the three ET isopeptides are encoded by distinct genes, production of ET-2 and ET-3 is mediated by a similar process to ET-1 (Figure 1) (20, 21).

Regulation of ET production

In vascular endothelial cells, mature ET-1 is continuously released through a constitutive pathway. The rate-limiting step of ET production is therefore thought to be at transcription of the prepro-ET-1 gene. Human prepro-ET-1 genes have five exons and a 5′-flanking region, spanning approximately 6.8 kb of DNA (Figure 2A) (22). Examination of regulation of ET-1 expression showed that transcription of prepro-ET-1 mRNA is regulated by various bioactive substances released by damaged tissues, including cytokines and hormones. Transforming growth factor β (TGFβ), thrombin, bradykinin, and tumor necrosis factor α (TNFα) stimulate transcription of the prepro-ET-1 gene in vascular endothelial cells (23–27). Some physiological and pathological conditions, such as hypoxia (28) and mechanical stress (29), also upregulate prepro-ET-1 mRNA levels. The ET-1 gene is under the transcriptional control of a TATA box at the position of 31 bases upstream from the transcription start site. Analysis of the 5′-flanking region revealed that the ET-1 gene has consensus sequences for binding of various transcription factors, including activator protein-1 (AP-1), GATA-2, Smad, hypoxia inducible factor-1α (HIF1α), and nuclear factor κB (NFκB) (30). These transcription factors coordinately couple intracellular signals triggered by extracellular stimuli to transcription of the prepro-ET-1 gene.

Inoue et al. reported that prepro-ET-1 mRNA in vascular endothelial cells is rapidly degenerated with a half-life of approximately 15 min (22). Expression of prepro-ET-1 mRNA is also regulated by alterations of mRNA stability. Human prepro-ET-1 mRNA has an AU-rich element (ARE) in the 3′-untranslated region (22), which is required for ARE-binding proteins to degenerate transcripts. ARE-binding proteins, such as AU-binding factor 1 (AUF1) and glyceraldehyde-3-phosphate dehydrogenase (G3PDH), induce a rapid degradation of prepro-ET-1 mRNA (31, 32). Regulation by AUF1 and G3PDH are suggested to be involved in ET-1 production induced by heat shock and oxidative stress, respectively (31, 32). Recent studies proposed that expression of prepro-ET-1 mRNA is also regulated by microRNAs (miRNAs). Analysis of the 3′-untranslated region of prepro-ET-1 mRNA revealed the presence of complementary binding sequences for miRNAs. Yeligar et al. found that overexpression of miR199 and miR155 in endothelial cells reduced expression of prepro-ET-1 mRNA (33).

ET receptor subtypes

Receptors for ETs are classified into ET_A and ET_B types (Figure 3). These ET receptors differ in their selectivity for ET ligands. ET_A receptors have ligand preferences of ET-1=ET-2>>ET-3, whereas ET_B receptors show equal selectivity for the three ET ligands. High expression of ET_A receptors is observed in the vascular smooth muscle and cardiocytes (4). Activation of vascular ET_A receptors is responsible for the potent vasoconstriction caused by ETs. ET_B receptors are abundantly expressed in the vascular endothelium, kidney, lung, and brain. Activation of ET_B receptors in endothelial cells causes vasodilatation by releasing nitric oxide (NO) and prostacyclins (34). ET_A and ET_B receptors are therefore suggested to have opposing roles in the regulation of vascular tone (35). Both ET receptor subtypes are G-protein-coupled receptors. Through G-protein-mediated mechanisms, ETs induce activation of phospholipase C and increased cytosolic Ca^{2+} (36), which are the main signaling pathways for the regulation of vascular tone. ETs also activate mitogenic and survival signals in various cell types. These signal molecules include mitogen-activated protein kinases (MAPKs), Akt, and Src (36). Daub et al. first reported that stimulation of ET receptors activates epidermal growth factor (EGF) receptors by EGF-independent mechanisms (37). Such 'transactivation' of growth factor receptors by ETs has been observed in glomerular mesangial cells (38), vascular smooth muscle cells (39), and ovarian cells (40). The transactivation is thought to be involved in the action of ETs as mitogenic and survival factors.

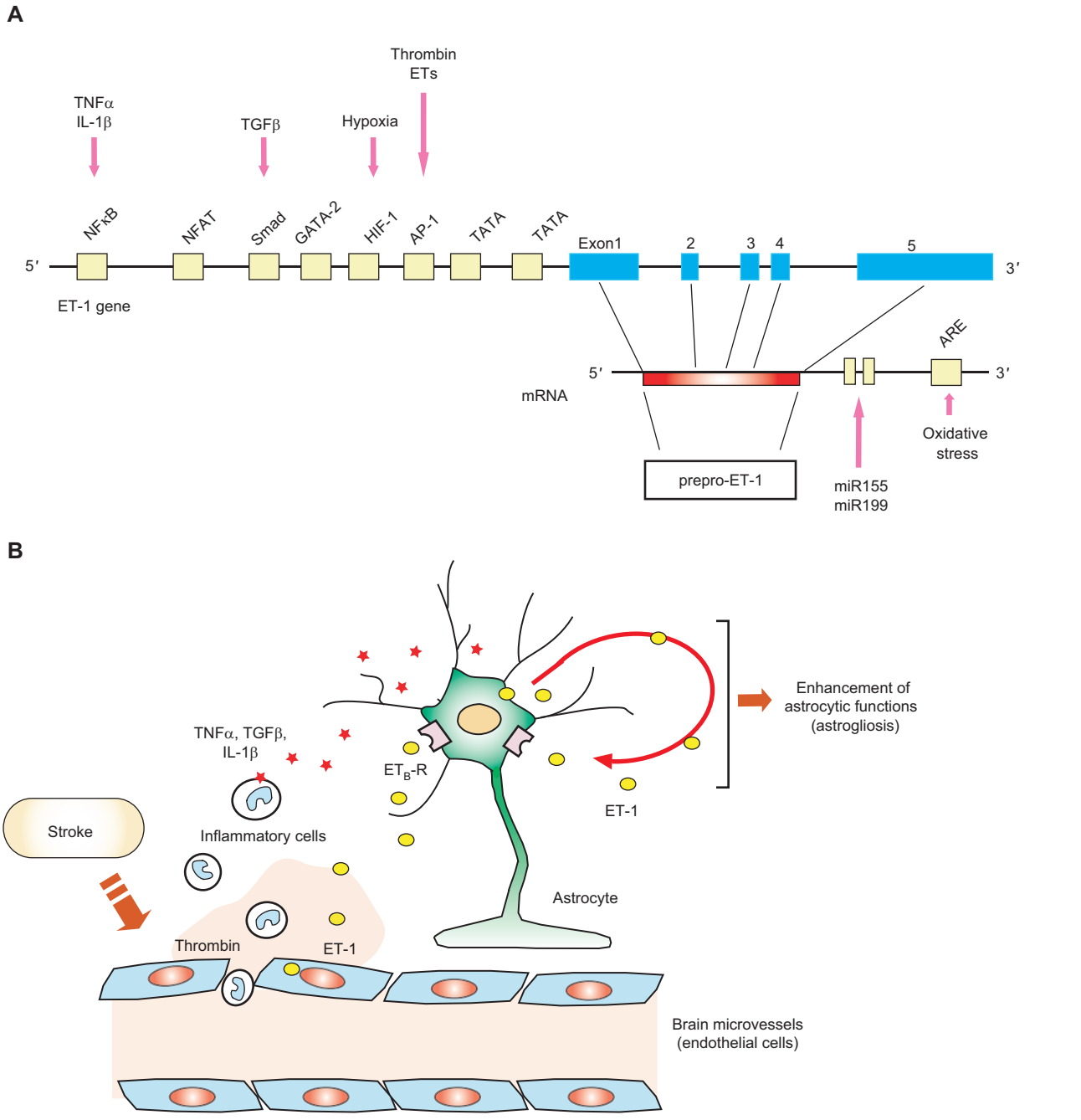

Figure 2 Signaling mechanisms stimulating ET-1 production.
(A) Transcriptional and post-transcriptional regulation of ET-1 gene expression. In the 5′-promoter region of the human ET-1 gene, recognition sites for several transcriptional factors, including NFκB, NFAT, GATA, HIFα, TATA, and AP-1, are present. Signaling molecules stimulating ET-1 production in vascular endothelial cells and astrocytes are indicated, along with transcription factors mediating their actions. In the 3′-untranslated region of prepro-ET-1 mRNA, an AU-rich element (ARE) and complementary sequences for microRNAs are present. Expression levels of prepro-ET-1 mRNA are also regulated by alteration of stability by ARE-binding proteins and microRNAs. (B) Expression of ET-1 in stroke. In the early stage of stroke, inflammatory cells enter nerve tissues across the disrupted blood–brain barrier. TNFα, IL-1β, and TGFβ are produced by blood-derived inflammatory cells. Increases in these cytokines, together with thrombin, stimulate ET-1 production in brain endothelial cells and astrocytes. Hypoxia following stroke induces brain ET-1 production. Increases in ET-1 stimulate several functions of astrocytes through ET_B receptors, including astrocytic ET production via an autocrine mechanism.

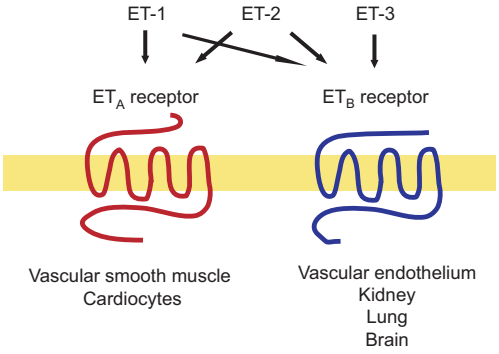

Figure 3 Ligand selectivity of ET receptor subtypes.
ET_A receptor subtype, which is predominantly expressed in smooth muscle cells and cardiocytes, has ligand selectivity for ET-1 and ET-2. ET_B receptors are expressed in the vascular endothelium, kidney, lung, and brain. Ligand selectivity of ET_B receptor is different from that of ET_A type; ET-1, ET-2, and ET-3 equally activate it.

The ET system in the central nervous system

ET production in the brain

Nerve tissues abundantly express ET-1 and ET-3 (6, 7). In normal brain, immunohistochemical observations have shown the presence of ET-containing neurons in the spinal cord (41), perivascular neurons of the basilar artery (42), and in the hypothalamic-neurosecretory system (43). ET-1 induces excitation of neurons in the spinal cord and trigeminal system (44, 45). These findings suggest roles for ETs in neurotransmission, which Dashwood and Loesch recently presented a detailed review (46). Increases in brain ETs are observed in nerve injury animal models (47, 48). In human brain, increased levels of ET-1 in cerebrospinal fluid (CSF) are reported in stroke, head trauma, and neurodegenerative diseases (8, 49, 50). Because of their increased production in brain disorders, pathophysiological roles of ETs have also been examined. Immunohistochemical observations of damaged brains have shown that ETs are produced by brain microvessel endothelial cells and astrocytes (9). Factors such as TNFα, interleukin-1β (IL-1β), and thrombin, as well as hypoxia, which results in brain damage, induce ET-1 production in brain microvessel endothelial cells (51) and astrocytes (52–54). ETs are also known to stimulate astrocytic ET-1 production (55). ET-induced ET production indicates that astrocytic ET production is potentiated by an autocrine mechanism, via astrocyte-derived ET-1 (Figure 2B).

ECEs in the brain

Big-ETs are cleaved by ECEs to become active ETs. There are two subtypes of ECEs: ECE-1 and ECE-2, which share 59% amino acid homology (56). ECE-1 is widely expressed in various tissues including nerve tissues. In contrast to the ubiquitous expression of ECE-1, ECE-2 expression is restricted to nerve tissues. Rodriguez et al. found high levels of expression of ECE-2 mRNA in the midbrain, pituitary, hypothalamus, and cerebellum of mammalian brains (57). ECEs can also cleave other bioactive peptides, such as neuropeptides and amyloid β (Aβ) proteins. Based on this substrate selectivity, roles for brain ECEs other than ET production have been proposed. Accumulation of Aβ proteins, components of the amyloid plaques observed in brains of AD patients, is considered to be a pathogenesis of AD. Deletion mutations of either ECE-1 or ECE-2 increase $Aβ_{1–40}$ and $Aβ_{1–42}$ proteins in mouse brain (58). ECE-2 knockout mice show impaired learning and memory (57). In addition, upregulation of ECE-2 expression is reported in brains of AD patients (59). On the basis of these findings, brain ECEs are thought to regulate Aβ protein turnover and be involved in the pathogenesis of AD (60). Neuropeptides such as bradykinin, neurotensin, and substance P are also substrates of ECEs. Roosterman et al. proposed a novel hypothesis that degradation of neuropeptides by ECEs in endosomes promotes recycling and resensitization of internalized neuropeptide receptors (61). These neuropeptides cleaved by ECEs cause neurogenic inflammation. In fact, inhibition of ECEs is shown to decrease substance P-induced plasma extravasation in rats, suggesting that ECE inhibitors impair neurogenic inflammation by reducing resensitization of neuropeptide receptors (62).

ET receptors in the brain

Both ET_A and ET_B receptors are present in the brain, although with different cellular distributions. Brain ET_A receptors are expressed in the vascular smooth muscle and mediate the potent vasoconstriction effects of ETs. Local application of ET-1 to the cerebral artery induced prolonged vasoconstriction and reduction of cerebral blood flow in rat and pig, through ET_A receptors (63, 64). Owing to the likelihood of hypoxia, reduction of cerebral blood flow often leads to brain dysfunction. Taking advantage of its vasoconstriction effect, application of ET-1 to animal brains is used as a model for ischemic brain injury (63). In several brain disorders, production of ETs is increased (7, 8). ET_A receptor-mediated reduction of cerebral blood

flow is believed to play a role in the aggravation of brain dysfunction caused by ischemia in many brain disorders (see below). In some populations of neurons, ET_A receptors have been shown to modulate neural transmission. For example, in isolated primary sensory neurons, ET-1 causes excitation and increases in cytosolic Ca^{2+} through ET_A receptors (65). Consistent with ET-induced excitation of sensory neurons, administration of ET-1 caused pain-like behavior in rats, and the effect was antagonized by BQ123, an ET_A antagonist (66). This action of ET-1 suggests a novel role of ETs, potentiating transduction of pain signals in somatosensory systems (67, 68).

ET_B receptors are the prominent type in the brain. High expression of brain ET_B receptors is observed in astrocytes (69–71). In addition, expression of astrocytic ET_B receptors is upregulated after brain injury (70), which indicates that the roles of ETs in astrocytes are more significant in brain pathologies. ETs are known to be a potent mitogen of astrocytes; in cultured astrocytes, ETs induce cell cycle progression through ET_B receptors (72, 73). Activation of ET_B receptors also causes morphological alterations, accompanied by cytoskeletal reorganization (74, 75) and reduction of gap junctional communication (76, 77). Astrocytes produce and release various bioactive substances, for example, neurotrophic factors, cytokines, chemokines, NO, and vascular permeability factors, through which they interact with neurons and brain microvessels (78). Production of astrocyte-derived molecules is stimulated by activation of astrocytic ET_B receptors (79). ET_B receptors are also expressed in other brain cells. Recent studies have proposed roles for ETs in the development of oligodendrocytes and neurons (80, 81). Nishikawa et al. reported high expression of ET_B in embryonic cortical neuronal progenitors (82).

Roles of ETs in brain pathology

Because expression of brain ET ligands and receptors increases in brain pathologies, many investigations of ETs have focused on brain disorders. There is accumulating experimental evidence that modulation of ET systems has beneficial effects on brain dysfunction in stroke and neurodegenerative diseases.

Vasospasm and brain edema

The concentration of ET-1 in CSF is increased after stroke (50, 83). Franceschini et al. reported a correlation between ET-1 concentration in CSF and brain infarct volume after subarachnoid hemorrhage (SAH) (84). Vasospasm of the cerebral artery often occurs after stroke. Because prolonged cerebral vasospasm aggravates ischemic brain damage, inhibition of vasospasm is a possible therapeutic strategy for neuroprotective drugs. In animal models of brain ischemia, ET_A receptor antagonists prevent reduction of cerebral blood flow and improve ischemic brain damage (85–88). These findings indicate that activation of brain ET_A receptors induces cerebral vasospasm in brain insults (Figure 4). After SAH and head trauma, vasogenic brain edema also occurs. Vasogenic brain edema is caused by influx of blood proteins across the disrupted blood-brain barrier (BBB). Accumulation of brain edematous fluid elevates intracranial pressure, which disrupts neuronal function and can result in death. In normal conditions, the BBB is maintained by low permeability of brain microvessel endothelial cells. The permeability of brain microvessels is not static, but is dynamically modulated by various permeability factors. In brain pathologies,

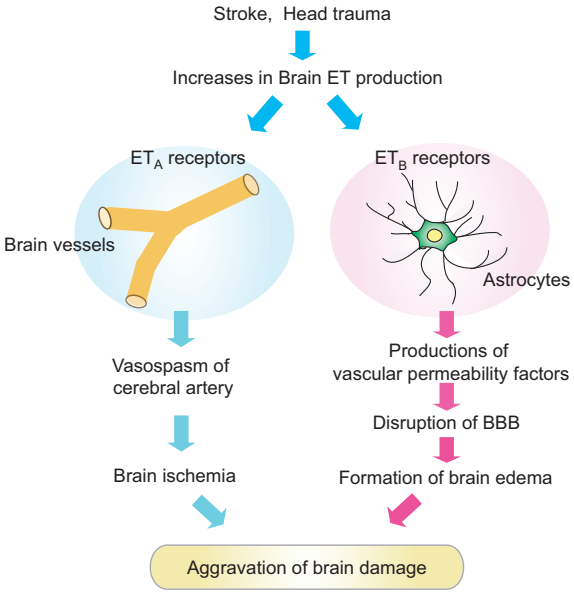

Figure 4 Involvement of ET receptors in vasospasm and brain edema formation after stroke.
In the early stage of stroke, increased ETs activate ET_A receptors in the brain vascular smooth muscle and induce vasospasm. Prolonged vasospasm leads to brain ischemia. ETs also stimulate ET_B receptors in astrocytes. Activation of ET_B receptors increases production of vascular permeability factors (VEGF and MMPs) in astrocytes. ET-induced production of astrocytic vascular permeability factors induces disruption of the BBB, which results in brain edema formation. Thus, in the early stage of stroke, ETs are thought to aggravate brain damage by inducing vasospasm and brain edema.

such as SAH and head trauma, the actions of permeability factors are increased. By excess actions of permeability factors, the barrier functions of brain microvessels are disrupted, which results in vasogenic brain edema (89). Vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs) are the main factors which increase permeability of vascular endothelial cells. In brain pathologies, astrocytic production of MMPs and VEGF is increased (90). ETs stimulate production of MMPs and VEGF in cultured astrocytes and rat brain through ET_B receptors (91–94). On the basis of these findings, ETs are proposed to be important factors in the development of vasogenic edema after stroke (Figure 4). Consistent with this idea, brain edema formation in rat models of brain ischemia and head trauma is reduced by ET antagonists (85, 95).

As described above, studies using animal models suggest that administration of ET antagonists has beneficial effects on neuronal damage in the acute phase of stroke. On the basis of these results in animal models, clinical trials of ET antagonists in SAH patients have been conducted. Clazosentan, an ET_A selective antagonist, was reported to reduce vasospasm and delayed ischemic neurological defects in SAH patients (96, 97). TAK-044, a nonselective ET antagonist, lowered the incidence of delayed neurological defects in SAH patients 3 months after the onset of symptoms (98).

Induction of reactive gliosis

In brain ischemia, head trauma, and neurodegenerative diseases, phenotypic conversion of astrocytes to the reactive type occurs, which is known as reactive gliosis (90). Reactive astrocytes are characterized by hypertrophy of the cell body and glial processes due to reorganization of cytoskeletal proteins. Conversion to reactive astrocytes is accompanied by functional alterations of astrocytes. Reactive astrocytes produce various bioactive substances, including neurotrophic factors, cytokines, chemokines, and proteases (78). Because these astrocyte-derived substances regulate neuronal viability, neuroinflammation, and repair of nerve tissues, preventing the induction of reactive astrocytes is a potential therapeutic target for many brain disorders. In addition to the enhanced production of bioactive substances, reactive astrocytes show a proliferative phenotype. Hyperplasia of reactive astrocytes leads to glial scar formation in damaged nerve tissues. Glial scars inhibit the repair of the damaged nervous system, by preventing axonal elongation and acting as a physical barrier to synaptogenesis. Administration of $Ala^{1,3,11,15}$-ET-1, an ET_B selective agonist, increased the number of reactive astrocytes in rat brain (99, 100). Induction of reactive astrocytes in stab wound brain injury was reduced by BQ788, an ET_B antagonist (101). These findings indicate that, in brain disorders, activation of astrocytic ET_B receptors is involved in phenotypic conversion to reactive astrocytes. Activation of ET_B receptors reproduces functional alterations of reactive astrocytes *in vitro*. Proliferation of cultured astrocytes was stimulated by activation of ET_B receptors (72, 73). In cultured astrocytes, ETs also induced reorganization of cytoskeletal proteins and altered morphology (74, 75), actions considered to be related to hyperplasia and hypertrophy of reactive astrocytes, respectively (79).

Expression of cyclin D proteins is increased in the late G1 phase and promotes G1/S phase cell cycle progression. Expression of astrocytic cyclin D1 and D3 was stimulated by activation of ET_B receptors (77, 102). Examination of ET_B receptor signals showed that different mechanisms play a role between astrocytic cyclin D1 and D3 expression. ET-induced astrocytic cyclin D1 expression was inhibited by disruption of cytoskeletal actin, suggesting an involvement of cell adhesion-independent mechanisms (102). In cell adhesion-independent mechanisms, extracellular signal-regulated kinases and protein kinase C mediated astrocytic cyclin D1 expression by ETs (102). By contrast, ET-induced expression of astrocytic cyclin D3 required integrity of cytoskeletal actin (77). ETs activated focal adhesion kinase (FAK), a key tyrosine kinase in cell adhesion-dependent proliferation, in cultured astrocytes (103). Expression of dominant-negative FAK mutants in cultured astrocytes prevented ET-induced G1/S cell cycle progression and cyclin D3 expression (102, 104). These findings indicate that ETs stimulate cyclin D3 expression and proliferation of cultured astrocytes through FAK-mediated adhesion-dependent mechanisms. Thus, ETs can activate both cell adhesion-dependent and -independent mechanisms in astrocytes. This cooperative action of two distinct signal pathways may underlie the potent mitogenic effects that ETs have on astrocytic proliferation.

Neuronal survival and neurogenesis

In addition to during development, the viability of neuronal cells in the adult brain is maintained by various trophic factors. A decline of these trophic signals increases the vulnerability of neuronal cells to other detrimental factors and cause neuronal death by apoptosis. Apoptotic neuronal death underlies the deficits in brain function in stroke and neurodegenerative diseases. In ET_B-deficient rats, increases in apoptotic neurons were

observed in the cerebellum (81) and dentate gyrus (105). Activation of ET_B receptors showed antiapoptotic actions in cultured neurons of the olfactory bulb and cerebrum (106–108). As for mechanisms underlying the antiapoptotic actions of ET_B receptor-triggered signals, inhibition of caspase-3 and voltage-dependent L-type Ca^{2+} channels was suggested (106–108). A histological observation on the dentate gyrus of rabbit and human meningitis by Ehrenreich et al. showed that increases in apoptotic death of dentate neurons were associated with reduction of neuronal ET_B receptors (105). These findings indicate that activation of ET_B receptors directly triggers trophic signals in some populations of neurons. In the hippocampus and subventricular zone of the lateral ventricle, new neurons are continuously generated from neural precursor cells. Recently, evidence has emerged that neurogenesis in the adult brain plays an important role in the repair of nerve tissues in brain disorders (109). Progenitors of cortical neurons show high levels of ET_B receptors. Activation of ET_B receptors in neural progenitors stimulated their proliferation and migration, indicating that ETs promote neurogenesis by directly acting on neural progenitors (82). These actions of ETs on neurons and neural progenitors suggest that activation of ET_B receptors promotes repair of nerve tissues in the chronic phase of stroke and neurodegenerative diseases (Figure 5).

Viability of neurons and repair processes of damaged nerve tissues are supported by neurotrophins and glial cell-derived neurotrophic factor (GDNF). Neurotrophin family proteins, including nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), which are produced by subpopulations of neurons in normal conditions, support the viability of cholinergic neurons in the basal forebrain, motor neurons, and hippocampal neurons (110). Recent studies showed that BDNF stimulates neurogenesis of the adult brain (111). GDNF supports survival of mid-brain dopaminergic neurons and has been suggested to have beneficial actions on patients with Parkinson's disease (110). In neurodegenerative diseases and stroke, production of these neurotrophic factors is increased, where astrocytes are the main source of neurotrophic factors (78). Because of their potent ability to support neuronal viability, potentiation of neurotrophic factor production has been a promising therapeutic strategy

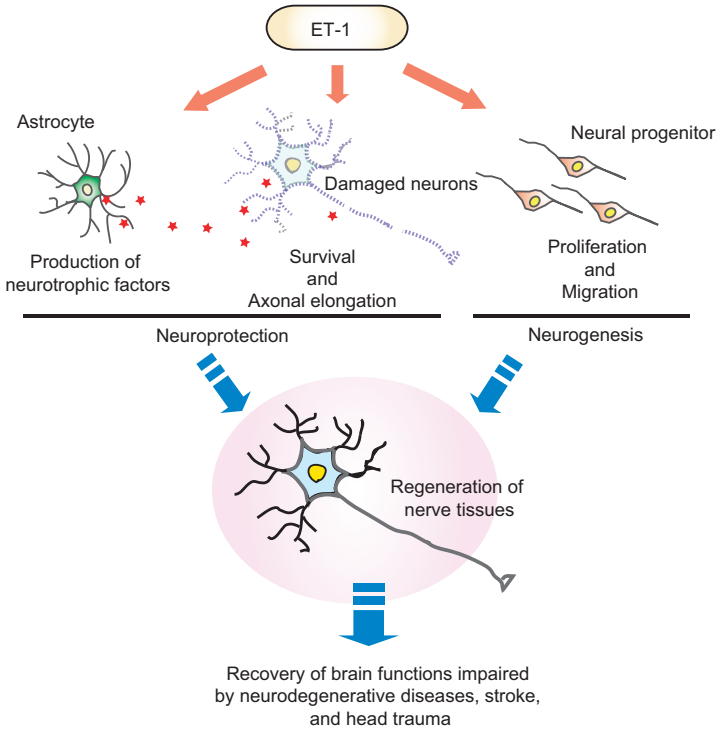

Figure 5 Possible roles of ET_B receptors in survival and regeneration of damaged nerve tissues.
In some neuronal cells, direct activation of ET_B receptors has been shown to induce antiapoptotic actions. Neuronal survival and regeneration is promoted by neurotrophic factors. Production of neurotrophic factors is stimulated by activation of astrocytic ET_B receptors. Neural progenitors express ET_B receptors. Proliferation and migration of neural progenitors are stimulated by ETs. These actions raise the possibility that activation of ET_B receptors promotes repair of damaged nerve tissues in neurodegenerative diseases and in the chronic phase of stroke.

for neuronal dysfunction in neurodegenerative diseases and stroke. Administration of $Ala^{1,3,11,15}$-ET-1 into rat brain increased production of NGF, BDNF, neurotrophin-3, basic fibroblast growth factor, and GDNF in reactive astrocytes (100, 112). The effects of ETs on neurotrophic factor production were also observed in cultured astrocytes (100, 112–115). As well as having direct actions on neurons (106–108), ET-induced neurotrophic factor production suggests a possible role of astrocytic ET_B receptors in supporting the viability and repair of damaged nerve tissues (Figure 5). A recent report by Leonard et al. showed that IRL1620, an ET_B agonist, reduced neuronal damage and neurological defects in a rat brain ischemia model (116).

Outlook

For several decades, neuroscientists and pharmacologists have invested much effort into the invention of novel drugs to provide protection or repair to nerve tissues impaired by brain disorders. However, at present, it is not possible to say that effective drug treatments for many neurodegenerative diseases and brain insults have been established. Recent studies of brain ETs have clarified the involvement of ET systems in various pathophysiological responses of damaged brains. As a target of neuroprotective drugs, brain ET receptors could have two possible clinical applications in different states of brain pathologies. One possible application is to block brain ET_A and ET_B receptors in the acute phase of stroke. Blockage of brain ET_A receptors ameliorates vasospasm of cerebral arteries and ischemic brain damage after stroke. In addition to ET_A receptors, blockage of ET_B receptors in astrocytes impairs brain edema formation by reduced production of VEGF and MMPs. The beneficial effects of ET antagonists have been shown in clinical trials for SAH patients. The other possible application of ET systems is to activate ET_B receptors in the chronic phase of brain insults and in neurodegenerative diseases. In these states, promotion of nerve repair processes, that is, axonal elongation, synaptogenesis, and neurogenesis, is therapeutic, to allow recovery of brain functions. Beneficial actions of ET_B agonists in chronic brain disorders can be supported by the findings that activation of brain ET_B receptors caused production of neurotrophic factors, antiapoptotic effects in neuronal cells, and proliferation of neuronal progenitors. Already, many agonists and antagonists for ET receptors have been developed. Thus, it is expected that a novel drug for treatment of brain disorders may be discovered among these ET receptor agonists and antagonists.

Highlights

- ET systems are involved in pathophysiological responses of damaged brain.
- Brain microvessels and astrocytes produce ETs in response to brain injury.
- ECEs cleave Aβ proteins and neuropeptides.
- Activation of ET_A receptors induces vasospasm of cerebral arteries after stroke.
- ET antagonists have beneficial actions on neurological defects in SAH patients.
- Activation of ET_B receptors in astrocytes promotes conversion to reactive astrocytes.
- Activation of ET_B receptors stimulates production of astrocytic neurotrophic factors.
- ETs stimulate proliferation and migration of neural progenitors.
- Brain ET systems are expected to be a novel target of neuroprotective drugs.

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