

Evidence for the endothelin system as an emerging therapeutic target for the treatment of chronic pain

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Abstract: Many people worldwide suffer from pain and a portion of these sufferers are diagnosed with a chronic pain condition. The management of chronic pain continues to be a challenge, and despite taking prescribed medication for pain, patients continue to have pain of moderate severity. Current pain therapies are often inadequate, with side effects that limit medication adherence. There is a need to identify novel therapeutic targets for the management of chronic pain. One potential candidate for the treatment of chronic pain is therapies aimed at modulating the vasoactive peptide endothelin-1. In addition to vasoactive properties, endothelin-1 has been implicated in pain transmission in both humans and animal models of nociception. Endothelin-1 directly activates nociceptors and potentiates the effect of other algogens, including capsaicin, formalin, and arachidonic acid. In addition, endothelin-1 has been shown to be involved in inflammatory pain, cancer pain, neuropathic pain, diabetic neuropathy, and pain associated with sickle cell disease. Therefore, endothelin-1 may prove a novel therapeutic target for the relief of many types of chronic pain.

Keywords: endothelin-1, acute pain, chronic pain, endothelin receptor antagonists

Discovery of endothelin-1 as an algogen

Endothelin-1 (ET-1) was discovered by its vasoconstrictive effects on pig arteries.^{1,2} It was thereafter isolated, cloned, and extensively studied as a new endogenous vasoactive peptide. Since then, the endothelin family has been implicated in several human pathological conditions, including congestive heart failure,³ hypertension, sepsis, asthma, renal failure, cerebral vasospasm, and neoplasia.⁴ Of great surprise, experimental administration of ET-1 in a human volunteer produced severe and long-lasting pain.⁵ This discovery created a paradigm shift in the investigation of ET-1 as not solely a vasoactive peptide, but potentially as an endogenous algogen important in the modulation of pain. Many pathological conditions in which pain is a major symptom show an elevation in plasma ET-1. This includes painful vaso-occlusive crisis and acute chest syndrome associated with sickle cell disease, Raynaud's disease, prostate cancer,⁶ breast cancer, and complex regional pain syndrome. Thus, there is growing interest in the endothelin family and its receptors as novel therapeutic targets for the treatment of pain.

Cellular and molecular biology of endothelin-1

ET-1 was named after the cells that were first known to produce it: endothelial cells. It is now recognized that ET-1 is produced by a variety of other cells including vascular smooth muscle cells,⁷ leukocytes, cardiac myocytes,^{8,9} mesangial cells,¹⁰ neurons,¹¹

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mast cells,¹² and macrophages.¹³ ET-1 belongs to a family of closely related peptides that include endothelin-2 (ET-2), endothelin-3 (ET-3), endothelin-4 (ET-4), and the sarafotoxins. These are all similar in structure to ET-1 but are separate gene products with tissue-specific expression.^{3,4}

Synthesis and release of ET-1 is modulated by both physiological and pathophysiological factors. Factors that have been shown to inhibit the production of ET-1 include nitric oxide, prostacyclin, and atrial natriuretic hormone.¹⁴ Factors that can induce the production of ET-1 include proinflammatory cytokines, growth factors, angiotensin II, norepinephrine, vasopressin, bradykinin, thrombin, mechanical stress, peripheral tissue injury, and hypoxia.^{15–17} In the vascular system, ET-1 is released from endothelial cells in a polarized fashion toward the smooth muscle interface.¹⁸ The elevation of plasma ET-1 levels following vascular injury is likely the result of spill-over from the smooth muscle compartment. ET-1 is thus acting as a local modulator of vascular tone, and not as a circulating hormone. ET-1 has a half-life of 7 minutes¹⁹ and is cleared from plasma after degradation by endopeptidases.^{20,21}

The endothelin family signals through the seven transmembrane G protein-coupled endothelin A (ET_A) receptor²² and the ET_B receptor.^{2,23,24} The two receptors can be differentiated by agonists and antagonists and in their cellular distributions (Table 1).²³ The ET_A receptor binds ET-1 with the greatest affinity,²⁵ whereas ET-1, ET-2, and ET-3 all have equal affinity for the ET_B receptor.²⁶ The ET_B receptor has further significance in ET-1 tissue clearance via agonist-dependent receptor internalization and lysosomal degradation.^{20,21}

The ET_A receptor couples to G_{q/11} and G_{q12/13} proteins, whereas the ET_B receptor couples to G_{i/o} and G_{q/11} proteins.²⁷ Both receptors mediate their actions through phospholipase C activation, which in turn activates inositol triphosphate and

diacylglycerol, causing a mobilization of calcium²⁸ and activation of protein kinase C (PKC),^{4,29} as well as PKC-independent signaling pathways.⁴

Clinical significance

ET-1 in the pain pathway

ET-1 and its receptors are found throughout the pain signaling pathway (Figure 1). In the periphery, ET_B receptors are expressed on endothelial cells,³⁰ smooth muscle cells,³¹ macrophages,³² and keratinocytes within the dermis and epidermis of the skin, where the peripheral endings of nociceptors are located. In contrast, ET_A receptors are expressed on the peripheral endings of the nociceptor themselves, as well as on nerve axons and the nociceptor cell bodies located in the dorsal root ganglion (DRG).³³ ET_A receptors have been found on small, medium, and large-sized nociceptors.³⁴ In contrast, in the DRG, ET_B receptors are found exclusively on satellite glial cells and Schwann cells that myelinate nociceptors, but not on the nociceptors themselves.^{33–35} ET_A and ET_B receptors are expressed in the DRG in a ratio of 60:40, respectively.³⁶

In humans, both small and large nociceptors also express endogenous ET-1 mRNA.³⁷ Brief exposure of nociceptors to ET-1 enhances action potential generation, possibly through decreasing delayed rectifier-type K⁺ currents.³⁸ In addition, ET-1 evokes hyperpolarizing shifts in tetrodotoxin-resistant sodium channels on nociceptors.^{28,39} Alteration in these specific channels is known to be involved in pain transmission caused by other algogens.⁴⁰ The shifts in the tetrodotoxin-resistant sodium currents may be enough to cause depolarization of sensory fibers and reduce the threshold for activation (hyperalgesia) of those fibers.³⁶

ET-1 mRNA is also found in laminae IV–VI in the spinal cord³⁷ and in a variety of brain regions associated with pain signaling, including the hippocampus, striatum, amygdala, hypothalamus, raphe nucleus, locus coeruleus, cerebral cortex, pontine tegmentum, and lateral reticular formation.^{41,42} Similarly, ET-1 protein is found extensively in laminae I–V,⁴³ and ET_A receptors have been found in the hypothalamus, reticular formation, pontine tegmentum, locus coeruleus, and substantia nigra.^{44,45} Thus, the endothelin system is located across multiple levels of the pain neuroaxis and participates in signaling cascades, the final effectors of which are key players in the physiology of acute pain.

ET-1 in acute pain

Peripheral administration of ET-1 produces pain and hyperalgesia in humans as well as paw flinching, licking, mechanical

Table 1 ET receptor agonists and antagonists commonly used in basic research and clinical studies

| Receptor | Agonists | Antagonists |
|--|---|--|
| ET _A receptor | ET-1 | BQ-123, atrasentan, SB234551, ABT-627, FRI39317, A-127722.5, YM598 |
| ET _B receptor | ET-1, ET-2, ET-3, IRL-1620, sarafotoxin S6c | BQ-788, A-192621 |
| ET _A /ET _B receptors | ET-1, ET-2, ET-3 | Bosentan |

Notes: ET-1 is listed as the only available agonist for the ET_A receptor. ET-2 and ET-3 will bind to the ET_A receptor, but they are not listed as ET_A agonists, as they have a 100-fold greater binding affinity for the ET_B receptor.²⁶ ET-1, ET-2, and ET-3 all have equal affinity for the ET_B receptor. IRL-1620 and sarafotoxin S6c bind exclusively to ET_B receptors. Several antagonists are listed for both receptors that are known to bind selectively to the receptor listed. Bosentan is the only known antagonist that has equal affinity for both the ET_A and ET_B receptors.

Abbreviation: ET, endothelin-1.

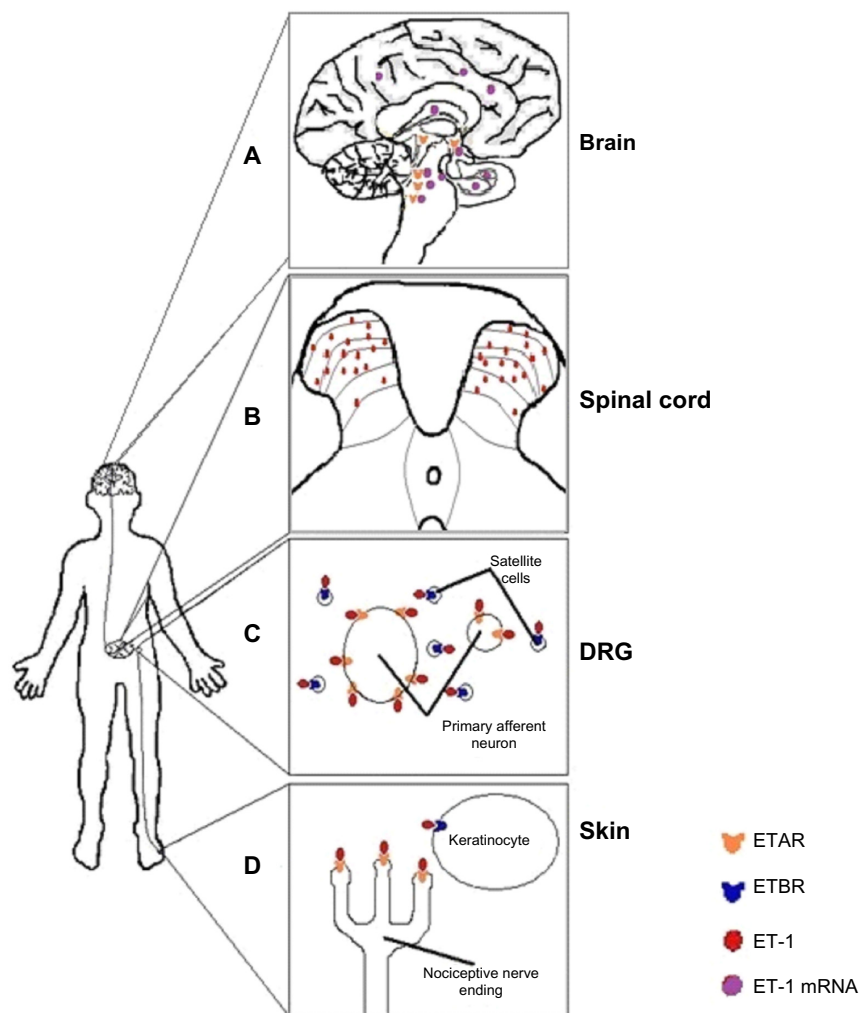


Figure 1 A schematic of the location of endothelin-1 (ET-1) and endothelin receptors found throughout the pain pathway.

Notes: ET_A receptor (ETAR) and ET_B receptor (ETBR) show different patterns of expression within the nervous system. Four areas are shown in detail in which ET receptors are known to be expressed on different cell types. **(A)** ET-1 mRNA and ETARs are found in several areas of the brain. ET-1 mRNA is found in the hippocampus, amygdala, hypothalamus, raphe nucleus, locus coeruleus, cerebral cortex, pontine tegmentum, and lateral reticular formation. The ETAR is also found in the hypothalamus, locus coeruleus, pontine tegmentum, and reticular formation, as well as the substantia nigra. **(B)** In the spinal cord, ET-1 immunoreactivity has been localized to laminae I-V. The expression of ET_A and ETBRs within the spinal cord laminae has thus far not been investigated. **(C)** In the dorsal root ganglion (DRG), ETAR immunoreactivity has been found on primary afferent sensory small and medium diameter neurons. ETBRs are localized on satellite cells and nonmyelinating Schwann cells. **(D)** Within the skin, ETARs are found on peripheral nerve endings in small- and medium-diameter neurons. ETBRs are found on endothelial cells, smooth muscle, and keratinocytes.

Abbreviation: ET, endothelin-1.

hyperalgesia/allodynia, and thermal hyperalgesia/allodynia in dogs, rats, and mice.^{46–49} ET-1 has been shown to directly activate nociceptors in humans and animals.^{50,51} In addition, ET-1 potentiates nociception induced by other algogens such as formalin, serotonin, and capsaicin.⁵² Similar to other algogens, central administration of ET-1 has opposite effects and produces antinociception.⁵³

Human studies

In human volunteers, ET-1 (125 μ g) administered into the brachial artery produced a sensation of heat, followed by vomiting and sweating and a deep muscular pain, with maximum severity at 2 hours and resolution at 10 hours; the pain

was intensified by touch and muscular contractions.⁵ Lower doses of ET-1 (12.5–50 μ g) administered intradermally into the forearm produced intense itching, erythema, increased sensitivity to pinching, and pressure tenderness that subsided after 2 hours.⁴⁶ In a similar study, intradermal administration of ET-1 (100 or 10 ng) resulted in spontaneous ongoing pain, with maximal pain score within 1 minute, and returned to normal after 30–60 minutes.⁴⁹ At these doses, tenderness to mechanical stimulation (von Frey filaments) and cold hyperalgesia developed and outlasted the observation period of 120 minutes; heat hyperalgesia did not develop.⁴⁹ Similar to intradermal, intracutaneous administration of ET-1 (25 ng–0.25 μ g) produced burning and stinging pain in

9 of 34 subjects, itch sensation in 11 of 34, and a mixture of both sensations in 14 of 34 subjects.⁵¹ These studies indicate that arterial administration of ET-1 produced long-lasting allodynia and deep muscular pain. Similarly, dermal administration of ET-1 produced itching and mechanical allodynia in low doses, whereas moderate doses produced spontaneous pain, mechanical hyperalgesia, and cold hyperalgesia (summarized in Table 2).^{46,49,51}

Single-fiber recordings from the peroneal nerve of human participants showed that ET-1 (25 and 2.5 ng) produced activity in 65% of mechanosensitive, but not mechano-insensitive, fibers with up to a third of the fibers having long-lasting responses (up to 15 minutes).⁵¹ Both fiber types showed sensitization to heat after ET-1 injection, with 62% of mechanosensitive fibers responding and 46% of mechano-insensitive fibers.⁵¹ It is also interesting that ET-1 produced a different pattern of activity compared with the algogens capsaicin and histamine. In contrast to the long-lasting responses in mechanosensitive fibers after ET-1 administration, capsaicin and histamine cause short bursts of activity in mechanosensitive fibers and longer-lasting activation patterns in mechano-insensitive fibers.⁵⁴ Thus, these studies suggest that ET-1 directly induces pain via mechanosensitive C-fibers and heat sensitization via both mechanosensitive and mechano-insensitive fibers. The firing pattern suggests that the mechanism of the pain-inducing actions of ET-1 is different from those of capsaicin and histamine.

Rodent studies: spontaneous nociceptive behaviors

As in humans, exogenous administration of ET-1 produces spontaneous pain-associated behaviors, as well as thermal and mechanical hyperalgesia and allodynia in rodents.

Table 2 Effect of endothelin-1 administration in humans

| Route of administration | Dose | Symptoms | Reference |
|-------------------------|----------|---|-----------|
| Intraarterial | High | Vomiting, sweating, deep muscular pain | 5 |
| Intradermal | Moderate | Intense itching, erythema, increased sensitivity to mechanical stimuli, pressure tenderness | 46 |
| Intradermal | Low | Spontaneous pain, increased sensitivity to mechanical stimuli, cold hyperalgesia | 49 |
| Intracutaneous | Low | Burning and stinging pain, itching | 51 |

Notes: Defined doses: high, >60 µg; moderate, 1–59 µg; and low, <0.9 µg.

Intradermal administration of high doses of ET-1 produces C and Aδ fiber activity as well as activity in a subpopulation of Aβ fibers.⁵⁰ In general, C and Aδ fibers relay nociceptive information, whereas Aβ fibers relay nonpainful tactile information. Flinching behaviors are absent when epinephrine (vasoconstrictor) alone is applied or when ET-1 is applied to surrounding muscle not innervated by the sciatic nerve.⁵⁵ This finding lends further support to ET-1-induced nociception being independent from its vasoconstrictor effects and is likely through direct activation of nociceptors. In addition to causing spontaneous behaviors after a single subcutaneous administration, a second administration of ET-1 1 day later produces desensitization in the same paw and sensitization in the contralateral paw, dependent on afferent transmission.⁵⁶

In the majority of studies, spontaneous ET-1-induced abdominal constrictions and ET-1-induced hind paw licking or flinching in the glabrous skin are mediated by the ET_A receptor.^{46,47,50,57–61} In contrast, ET-1 administration into the hairy skin induces transient antinociception mediated by the ET_B receptor.⁶²

Species differences appear when examining the role of ET_B receptors in ET-1-induced nociception. In mice, ET_B receptors have no effects in ET-1-induced licking behavior.^{58,60} In the mouse cheek model, ET-1 causes both pruritic and nociceptive behaviors, and injection of an ET_B antagonist causes potentiation of these behaviors, whereas coinjection of an ET_A antagonist with the ET_B antagonist significantly attenuates both behaviors.⁶³ In contrast, in rats, agonists of the ET_B receptor have antinociceptive effects, which are dependent on endogenous opioid release.^{50,59,61,64} Activation of ET_B receptors on keratinocytes induces the release of β-endorphins, which activate μ-opioid receptors on nearby nociceptors, causing hyperpolarization and, ultimately, a decrease in nociception (Figure 2).⁶⁴

Rodent studies: thermal and mechanical hyperalgesia

Similar to studies in humans, low doses of ET-1 produce a localized mechanical hyperalgesia in rodents.^{46,65,66} At higher doses, ET-1 produces a systemic mechanical hyperalgesia in both the ipsilateral and contralateral hindpaws.⁵⁶ ET_B receptor activation mediates the mechanical hyperalgesia induced by high doses of ET-1, which is in contrast to the analgesic activity of ET_B receptor activation in ET-1-induced spontaneous nociception.^{67,68} In mouse models of inflammatory pain, ET_A receptors are involved in both thermal and mechanical hyperalgesia, whereas ET_B receptors are only involved in mechanical hyperalgesia.⁶⁷ It has also

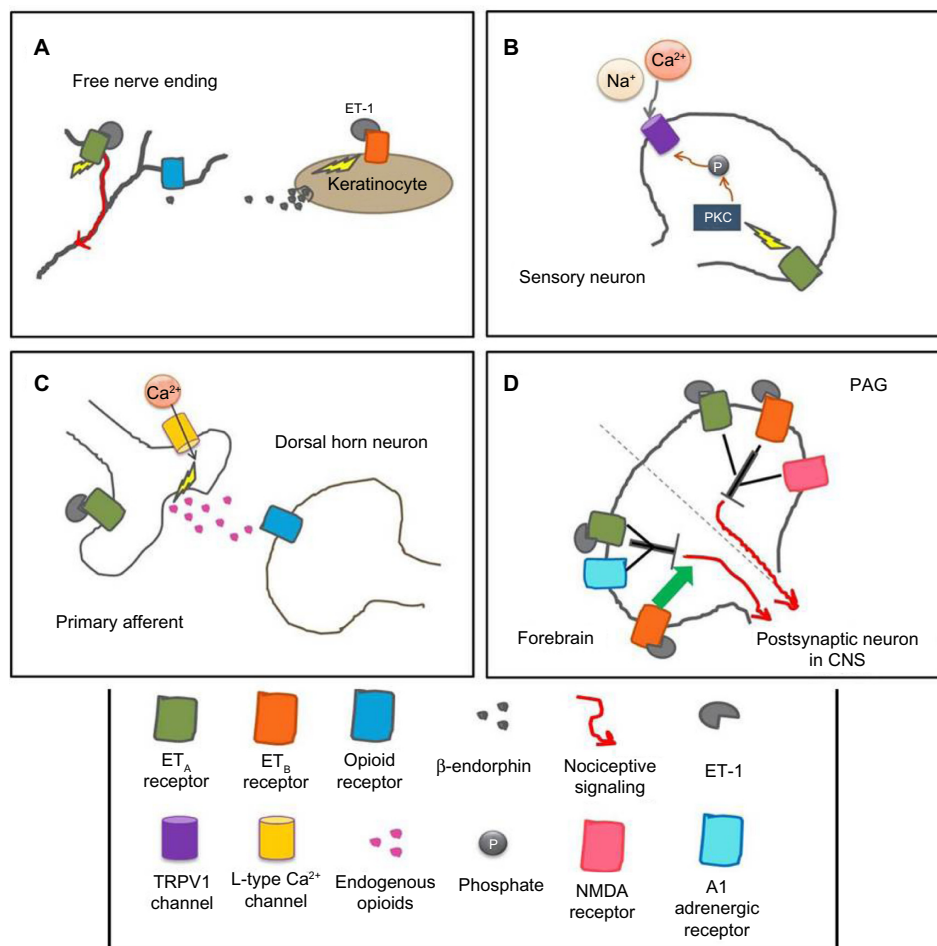


Figure 2 Mechanisms of the endothelin system in various parts of the pain pathway at the cellular level in rodents.

Notes: (A) Endothelin-1 (ET_A) receptors located on nociceptors located in the skin mediate nociception; however, ET_B receptors located on keratinocytes mediate antinociception by causing the release of β -endorphin from keratinocytes, which activate opioid receptors on nociceptors, thereby decreasing the signal. (B) When a sensory neuron is stimulated by capsaicin and ET-1, ET_A receptors activate protein kinase C, which causes the phosphorylation of transient receptor potential vanilloid subfamily channels, thereby allowing cations to flow through the channel, causing potentiation of the signal. (C) Intrathecal administration of ET-1 causes the activation of L-type Ca^{2+} channels and the release of endogenous opioids, thereby causing analgesia. (D) ET-1 administration into the PAG produces analgesia via the ET_A and ET_B receptors and is dependent on N-methyl-D-aspartate activation. An intracerebroventricular injection of ET-1 also elicits analgesia; this is mediated by the ET_A receptor and $\alpha 1$ adrenergic receptor and antagonized by the ET_B receptor.

Abbreviations: CNS, central nervous system; TRPV1, transient receptor potential vanilloid subfamily; NMDA, N-methyl-D-aspartate; PAG, periaqueductal gray; ET, endothelin-1.

been shown that calcitonin gene-related peptide (CGRP) and N-methyl-D-aspartate receptor antagonists can attenuate ET-1-induced mechanical sensitization.⁶⁹ In addition, the mechanism involved in this mechanical hyperalgesia appears to be independent of ET-1-induced vasoconstriction, sympathetic nervous system activation, and prostaglandins.⁶⁵

In humans, intradermal ET-1 induces thermal hyperalgesia by enhancing sensitivity of C-fiber nociceptors to heat.⁵¹ Similarly in mice and rats, low doses of ET-1 have been reported to produce thermal hyperalgesia.^{56,60} Studies with ET_A and ET_B receptor antagonists generally support a prohyperalgesic role of the ET_A receptor in thermal hyperalgesia and an unclear role for the ET_B receptor.^{60,70,71} However, ET-1-induced thermal hyperalgesia occurs similarly in both conditional ET_A knockout mice and control mice.⁷² This

suggests that thermal hyperalgesia occurs independent of ET_A receptors while highlighting the need for further study.

The transient receptor potential vanilloid subfamily (TRPV1) channel may play a role in ET-1-induced thermal hyperalgesia. In mice with conditional deletion of the ET_A receptor on sensory neurons, thermal and mechanical sensitivities were unaffected; however, capsaicin-induced nocifensive behaviors were significantly reduced.⁷² TRPV1 channels are colocalized with ET_A receptors on sensory neurons in the DRG, and both endothelin receptors are co-localized with TRPV1 channels on neurons in the rat trigeminal ganglia.^{34,73} ET-1 potentiates capsaicin-induced TRPV1 currents via activation of PKC ϵ .^{73,74} Therefore, activation and sensitization of nociceptors by ET-1 may be mediated by ET_A receptors activating PKC ϵ , which targets

TRPV1 channels (Figure 2). In support of this mechanism, TRPV1 knockout mice show reduced ET-1-induced thermal hyperalgesia.⁷⁰ In addition, in HEK293 cells expressing both ET_A and TRPV1 channels, ET-1 evokes inward current responses, which are not seen in cells expressing only ET_A receptors. In HEK293 cells and in the skin, ET-1 leads to the phosphorylation of TRPV1.⁷⁰ Together, these results suggest there is an interaction between ET_A and TRPV1 channels that may have pronociceptive effects.

Rodent studies: potentiation of algogens

Formalin, capsaicin, and serotonin are algogens that induce spontaneous nociceptive behaviors when administered subcutaneously in rodents. In rats and mice, ET-1, through ET_A and ET_B receptors, potentiates formalin-induced nociception; this effect is also seen contralaterally in rats.^{52,56,71} In addition, ET-1 potentiates capsaicin-induced nociception via the ET_A receptor⁷⁵ and enhances capsaicin-stimulated release of CGRP in sensory neurons and c-Fos expression in the dorsal horn of the spinal cord.^{69,76} Serotonin-primed capsaicin-induced nociception is abolished by administration of high doses of ET-1 or an ET_B receptor agonist.⁷⁵ The general hypothesis is that at lower ET-1 doses, the ET_A receptor potentiates capsaicin-induced hyperalgesia, but at higher ET-1 doses, the ET_B receptor mediates an antinociceptive effect. This is similar to the working model, in which a high dose of ET-1 induces algosia via ET_A receptors and a concurrent analgesic effect mediated via ET_B receptors.⁵⁵

Rodent studies: acute postsurgical pain

The endothelins have been implicated in postsurgical pain, using a rat model of postincisional pain in which both primary and secondary mechanical allodynia and hyperalgesia develop.⁷⁷ The ET_A receptor mediates primary and secondary mechanical allodynia. In contrast, the ET_B receptor mediates secondary hyperalgesia, but not primary hyperalgesia.⁷⁷ These findings suggest that local blockade of ET_A receptors before incision may be a viable therapeutic approach for reducing postincisional pain.

ET-1 in the central nervous system

In contrast to peripheral studies showing ET-1 as an algogen, administration of ET-1 into the central nervous system is often antinociceptive. Intrathecal ET-1 produces dose-dependent thermal analgesia in mice that is reduced by the nonspecific opioid receptor antagonist naloxone or a δ -opioid receptor antagonist.⁷⁸ The analgesic actions of intrathecal ET-1 administration occur through activation of L-type Ca²⁺

channels and the release of endogenous opioids (Figure 2).⁷⁸ Although ET-1 administered peripherally enhances formalin-induced nociception, intrathecal ET-1 decreases formalin-induced nociception via the ET_A receptor.⁷⁹ Similarly, transgenic mice that overexpress ET-1 in astrocytes have a significant decrease in nociceptive behaviors during the second phase of the formalin test compared with non-transgenic mice, further supporting an antinociceptive role for ET-1 in the spinal cord.⁸⁰

Administration of ET-1 into the periaqueductal gray (PAG) of mice produces thermal analgesia mediated by both the ET_A and ET_B receptors and dependent on N-methyl-D-aspartate activation (Figure 2).^{53,81} Intracerebroventricular ET-1 dose-dependently produces analgesia to thermal stimuli,⁸² which is mediated by the ET_A receptor and the α 1 adrenergic receptor and antagonized by the ET_B receptor (Figure 2).⁸² Thus, ET-1's analgesic actions in the brain may be mediated via ET_A and ET_B receptors through a noradrenergic pathway that activates a descending inhibitory pathway to the spinal cord, possibly through the release of endogenous opioids.

Age and sex differences

Cardiovascular studies suggest that the biological activity of ET-1 in humans varies depending on age, sex, and concentration of ET-1. Some of this variability results from sex- and age-dependent expression of ET_A and ET_B receptors. In the saphenous vein, postmenopausal women demonstrate a 1:1 ratio of ET_A:ET_B expression compared with age-matched male ratios of 3:1 expression.⁸³ Age-specific expression patterns have also been demonstrated, with infants having higher peripheral levels of ET-1⁸⁴ and a higher density of endothelin receptors in the heart compared with older children and adults,⁸⁵ suggesting a decrease in ET-1 activity after infancy.

Similarly, adult rodents show sex differences in the onset of ET-1-induced mechanical hyperalgesia, with a more rapid onset of hyperalgesia in adult males and ovariectomized females when compared with intact females.⁸⁶ In addition, male and ovariectomized female rats, but not intact females, show progressively increased hyperalgesia after repeated mechanical stimulation. In neonatal rats, ET-1-induced spontaneous licking and flinching behaviors are age- and sex-dependent.⁶¹ ET-1-induced nociceptive behaviors are significantly greater in younger animals and decrease with age. At early ages, males show increased nociception compared with females. The sex difference in ET-1-induced spontaneous nociception decreases with maturation. Similarly, an age- and sex-dependence was observed for the priming effect

of ET-1 on subsequent ET-1 exposures, which is important in certain disease states in which there are repeated exposures to ET-1, such as sickle cell disease. Exposure to ET-1 during the neonatal period produced an increase in nociceptive behaviors in response to a second exposure to ET-1 4 days later in male rats.⁸⁷ In contrast, a priming exposure to ET-1 in neonatal female rats decreased nociceptive behaviors when exposed to a second administration of ET-1.⁸⁷ Furthermore, sensitization to a second ET-1 exposure was also observed in 2-month-old male rats, but the desensitization to a second ET-1 exposure was not observed in older females. This suggests a long-lasting sensitization in male rats exposed to ET-1 in the neonatal period and a short-lasting desensitization in female rats. A subsequent study showed that a first exposure to ET-1 at 3 weeks after birth did not show the same priming to a second exposure to ET-1 as found in the neonatal rats, suggesting a window of vulnerability for ET-1-induced priming of nociceptive responses.

Mechanistically, ET-1-induced priming in neonatal males decreased ET_B receptor expression in the skin and sensitized the rats to subsequent ET-1 administration. In neonatal females, ET-1-induced priming increased ET_B receptor expression in the skin and desensitized the rats to subsequent ET-1 administration. No changes in ET_A or μ -opioid receptor expression were found.⁸⁷ Furthermore, ET-1-induced sensitization in neonatal males could be prevented with morphine administration at the time of the priming dose of ET-1, which prevented the downregulation of ET_B receptor expression. These studies highlight the importance of the ET_A-to-ET_B receptor ratio in the modulation of ET-1-induced pain.

ET-1 in inflammatory pain

Endogenous ET-1 is implicated as a mediator in inflammatory pain. ET-1 is released during the inflammatory process, and its production is stimulated by the inflammatory mediators interleukin 1 β , tumor necrosis factor α , and tumor necrosis factor β .^{88,89} In addition, human neutrophils synthesize ET-1, providing an additional source of ET-1 in inflammatory states.⁷⁷ Both acute (carrageenan) and chronic (complete Freund's adjuvant) inflammatory rodent models produce thermal and mechanical hyperalgesia that is modulated by endogenous ET-1 signaling. Thermal hyperalgesia in these mouse models appear to be primarily modulated by ET_A receptors.⁶⁷ However, in a rat complete Freund's adjuvant model, both endothelin receptors appear to be involved in modulating thermal hyperalgesia with a more pronounced role for the ET_B receptor.⁹⁰ Both endothelin receptors are also involved in modulating carrageenan- and complete

Freund's adjuvant-induced mechanical hyperalgesia.^{67,90} One theory for the role of the ET_B receptor in inflammatory pain is that activation of the ET_B receptor causes the release of tumor necrosis factor α and interleukin 6, which activate nociceptors to produce mechanical hyperalgesia. Nociception induced by ET-1 in inflammatory pain is mediated via different receptors, depending on the type of inflammation.^{91,92} In the naïve rat joint, ET_A receptors mediate inflammation; in the carrageenan-primed joint, both endothelin receptors are involved; and in the carrageenan-primed joint challenged with lipopolysaccharide, the ET_B receptor mediates the effects. These studies show that the interaction between inflammation and the endothelin system is highly complex and depends on the specifics of the inflammation.

One common inflammatory pain condition is arthritis, and it has been shown that ET-1 plasma levels are increased in patients with rheumatoid arthritis and osteoarthritis compared with control patients.⁸⁹ ET-1 levels are also higher in patients with active rheumatoid arthritis compared with those with inactive rheumatoid arthritis.⁸⁹ In a mouse model of antigen-induced arthritis, both the genes encoding for ET-1 and the ET_A receptor were upregulated in lumbar DRGs.⁹³ A mixed ET_A/ET_B receptor antagonist inhibited acute and chronic inflammation during antigen-induced arthritis flare-up reactions. Similarly, in an ovalbumin-induced mouse model of chronic arthritis, a mixed ET_A/ET_B receptor antagonist or a selective ET_A receptor antagonist reduced nociceptive behavior, whereas a selective ET_B receptor antagonist enhanced nociception.⁹⁴ These studies suggest that the pain caused by arthritis may be alleviated via ET receptor antagonists; however, the dichotomy of ET_B receptor actions in these models remains to be elucidated.

Specific diseases

Cancer

ET-1 protein or mRNA is hypersecreted by many cancer types, such as prostate, breast, pancreatic, colon cancer, and human oral squamous carcinoma cell lines.^{4,95,96} ET-1 is believed to be involved in many aspects of cancer progression, such as tumor growth and cell proliferation.⁶ Pain is prevalent in about 70% of people with metastatic cancers such as prostate and breast cancer.⁹⁷ Exogenous application of ET-1 in animal models of cancer has been found to potentiate cancer-induced nociception and sensitization of C-fibers to heat via activation of ET_A receptors, a similar pathway as that seen in ET-1-induced potentiation of formalin- and capsaicin-induced nociception.^{98,99} It has been shown that the concentration of ET-1 has a more direct correlation to

pain levels than the size of the tumor in mice,¹⁰⁰ illustrating the importance of evaluating algogens released by cancerous tumors, such as ET-1, to identify novel therapeutic targets to treat cancer pain.

Broadly speaking, there are two categories of endothelin-derived tumors: those that hypersecrete ET-1, upregulate ET_A receptors, and moderately downregulate ET_B receptors (colon, ovarian, pancreatic, prostate, and renal cell carcinoma), and those that hypersecrete ET-1, upregulate ET_B receptors, and moderately downregulate ET_A receptors (lung and breast).⁶ Several types of cancer cells are known to produce ET-1 in culture or after injection into animal models, as indicated by increased ET-1 protein and/or mRNA levels.^{96,101–104} These high levels of ET-1 seem to correlate with increases in nociceptive behaviors, mechanical hyperalgesia, and mechanical allodynia, which are mediated by ET_A receptors.^{72,101–104} In murine models of cancer pain, thermal hyperalgesia is significantly increased, which may be mediated by ET_B receptors in the early stage and the ET_A receptor in long-lasting hyperalgesia.^{96,105} Clinical studies using the ET_A receptor antagonist atrasentan have already shown some promise in improving cancer-related pain.⁴ These results suggest that targeting the endothelin receptors may prove a novel pain-reducing therapy in cancer pain.

Diabetes

Patients with diabetes often develop sensory neuropathy characterized by alterations in their ability to sense the environment. Diabetic neuropathy can manifest as a painless syndrome, in which there is a loss of touch, temperature, and pain sensation, or as a painful syndrome, in which thermal, mechanical, and tactile hyperalgesia and/or allodynia are present.³⁵ The ET-1 system has been affected in diabetic neuropathy in experimental animals. In a model of type 1 diabetes using streptozotocin (STZ), plasma ET-1 immunoreactivity is significantly increased and ET_B receptors have an abnormal appearance on satellite cells, in addition to a decrease in expression.^{35,106} STZ-treated animals and transgenic rats (DBH-ET_B:ET_B^{sl/sl}) exhibit significant increases in both mechanical hyperalgesia and tactile allodynia.³⁵ Tactile allodynia of STZ-treated rats can be reduced with acute or chronic inhibition of ET_A receptors, but not ET_B receptors alone or effectively with inhibition of both receptors.¹⁰⁷ Intrathecal administration of ET-1 produces antinociception in control but not STZ-treated mice, unless the dose of ET-1 is reduced, which produces dose-dependent antinociception in STZ-treated but not control mice.¹⁰⁸ This suggests there may be an upregulation of endothelin receptors in the STZ

model of diabetic mice. ET-1 and its receptors are implicated in diabetic neuropathies, as suggested in the aforementioned animal models. The dichotomy of endothelin receptors still remains to be fully characterized, and further investigation is needed. Use of endothelin receptor antagonists in the treatment of diabetic pain is yet to be researched in humans.

Neuropathic pain

Neuropathic pain is a type of chronic pain in which nerve damage has occurred, and it is often difficult to diagnose and treat effectively. The endothelin system has been implicated in being directly involved in the development of neuropathic-like pain in animal models. In a model of trigeminal neuralgia, inducing injury to the trigeminal nerve results in mechanical allodynia that is only reduced by ET_B receptor antagonism, as opposed to an ET_A or mixed receptor antagonist.¹⁰⁹ Cold hyperalgesia also develops in this model, and this effect is suppressed by both ET_B and ET_A receptor antagonists.¹¹⁰ In a mouse model of complex regional pain syndrome type 1, ET-1 administration causes hypersensitivity and an ET_A receptor antagonist reduces ET-1-induced sustained nociceptive behaviors, whereas an ET_B receptor antagonist enhances those behaviors.¹¹¹ In the chronic constriction injury and spinal nerve ligation (SNL) peripheral nerve injury models, the ET_A and both ET_A and ET_B receptors, respectively, play important roles in the development of nociceptive-associated behaviors.^{112,113} In the chronic constriction injury model, ET-1 mRNA and ET_A receptor mRNA and protein are increased in the nerve and at the site of injury.¹¹² In the SNL model, both ET_A and ET_B receptor proteins are increased in the injured nerve.¹¹³ In rats with SNL, injection of ET-1 into the hind paw causes significantly greater nociceptive behaviors compared with sham animals, which are attenuated by an ET_A receptor antagonist. SNL also causes an enhancement of ET-1-induced increases in intracellular calcium in neuronal cells, which may help explain the increase in nociceptive behaviors that develops after ET-1 administration in those animals.¹¹³ These studies suggest the endothelin system may provide an effective target in pain therapy for neuropathic pain conditions.

Gastrointestinal disorders

The endothelin system has been implicated in playing a role in some forms of visceral pain. In mice, an intraperitoneal injection of ET-1 causes rapid onset of abdominal constrictions.¹¹⁴ Mice lacking either one or both copies of the ET_B receptor gene have significantly reduced or absent abdominal constrictions in response to phenylbenzoquinone, which is used to elicit overt nociception in

the abdomen.¹¹⁵ The endothelin system is also known to play an important role in the development of the enteric nervous system. When the ET-3 or ET_B receptor gene is deleted, mice develop a condition similar to Hirschsprung's disease in humans, in which the enteric nervous system fails to innervate the colorectum, leading to distension of the bowel.¹¹⁶ These mice also display a lack of nociceptive response to distension of the rectum compared with wild-type mice, which is thought to be a result of an impairment in the signaling from low-threshold, wide-dynamic range afferents from the rectum.¹¹⁶

In humans, ET-1 has been found to be elevated in patients with active inflammatory bowel disease,¹¹⁷ Crohn's disease, and ulcerative colitis,^{118,119} which are gastrointestinal conditions known to have pain as a major symptom, compared with controls. Although the endothelin system has not been directly linked to the pain associated with these conditions, it may be worth exploring, as ET-1 has been implicated in other inflammatory pain conditions.

Sickle cell disease

Children and adults with sickle cell disease (SCD) experience recurrent, unpredictable painful vasoocclusive episodes. The endothelin pathway has been implicated in this type of painful episode, both as a trigger in the onset of vasoocclusive episodes and as a direct activator of nociceptors.^{57,114} Red blood cells and sickled red blood cells from homogenous sickle cell patients cause an increase in the release of ET-1 and the induction of ET-1 mRNA, respectively, from endothelial cells, suggesting ET-1 may be an important factor in facilitating vasoocclusive episodes.^{120,121} In endothelial cells exposed to plasma from SCD patients at different disease stages, there is an increased production of ET-1 during acute chest syndrome, with the highest levels of ET-1 production occurring with samples that were taken 4 days before hospital admittance for vasoocclusive episodes.¹²² Increased circulating ET-1 levels have been demonstrated clinically in sickle cell patients during vasoocclusive episodes.^{122–126} Hydroxyurea is used in the treatment of painful vasoocclusive episodes, and it has been shown to downregulate ET-1 gene expression in endothelial cells.^{127,128} Children with SCD treated with hydroxyurea have been shown to have levels of circulating ET-1 that were two times lower than those of untreated SCD children or controls.¹²⁹ Current research is being conducted to determine the relationship of the endothelin pathway and painful vasoocclusive episodes.

Vasoocclusive pain has been modeled in rats by injecting endothelin in the hind paw. One hypothesis is that prior

exposure to ET-1 will alter behavioral responses to subsequent ET-1 administration in a sex-specific manner. Young male rats become sensitized to pain after being "primed" with ET-1, whereas females appear to become desensitized to pain after being primed with ET-1.⁶¹ Additional results suggest that these differences between males and females may be a result of changes in the ET_B receptor.⁸⁷ In a mouse model of SCD, the ET-1 gene has been found to be upregulated after chronic exposure to hypoxia, and a mixed ET_A/ET_B receptor antagonist can prevent hypoxia-induced vasoocclusive episodes.^{130,131} Clinical studies have also been done to examine correlations between ET-1 levels and pain levels. Elevated plasma levels of ET-1 have been found in symptomatic SCD patients, and these levels increase with increased pain ratings.¹²⁴ In these patients, ET-1 levels decreased with a decrease in pain levels and a subsiding of symptoms but did not reach levels seen in healthy controls. Similarly, high plasma levels of ET-1 and Big Endothelin, the precursor of ET-1, in children with SCD were positively correlated with higher baseline pain ratings and greater baseline pain responses, respectively, before venipuncture.¹³²

In addition to contributing to painful episodes in SCD, the endothelin system may also be involved in the sickling of red blood cells and other complications of SCD. ET-1 has been shown to play a role in modulating the activity of the Gardos channel, which is important in sickle erythrocyte dehydration, in erythrocytes via the ET_B receptor.^{133–135} A polymorphism in the gene encoding for ET-1 is associated with the occurrence of vasoocclusive crisis and acute chest syndrome, a common complication of SCD, in children with SCD.¹³⁶ A disruption of nitric oxide homeostasis has been implicated in SCD; one of its many consequences is an increase in ET-1.^{137–139} Nitric oxide that is derived from endothelial cells regulates ET-1 expression, which contributes to the modulation of vessel tone.¹³⁹

These studies expose the need for a better understanding of the role of the endothelin system in SCD and the pain associated with it, so that alternative treatments for SCD can be explored.

Morphine analgesia/tolerance

Endothelin antagonists have not only been found to be analgesic themselves but have also been shown to enhance the analgesia of morphine. Centrally administered ET_A receptor antagonists have been shown to increase morphine analgesia.¹⁴⁰ Similarly, centrally administered ET_A receptor antagonists may reverse opioid tolerance and rescue opioid analgesia.¹⁴⁰ In contrast, an ET_B receptor agonist does not

increase morphine analgesia.¹⁴¹ These studies suggest that centrally administered ET_A receptor antagonists may enhance and extend the time of morphine analgesia, in addition to decreasing morphine tolerance. Thus, ET_A receptor antagonists may not only reduce pain experienced but may also aid other analgesics in combination therapies.

Implications to human disease and conclusions

The current research investigating the nociceptive effects of ET-1 in rodent models all concur that ET-1 receptors may prove a novel target for pain-relieving therapies. Studies using antagonists have shown that activation of ET_A receptors in the periphery is pronociceptive (Table 3); therefore, blocking this receptor would provide pain relief. Identifying the actions of the ET_B receptor presents a more challenging feat (Table 1). The effects of the receptor appear to be pronociceptive in mediating mechanical hyperalgesia in low and high doses.^{46,65,68} Conversely, this receptor appears to be analgesic when high doses of ET-1

are administered in the vicinity of keratinocytes.⁵⁹ In other animal models, it appears that activation of the ET_B receptor has no effect on nociception (Table 3).^{58,60} These seemingly contradictory findings may be the result of differences in the species used, sex differences, ET-1 doses, nociceptive testing procedures, or other such caveats. Therefore, further studies are needed to elucidate the value of selective ET_B receptor antagonists and agonists in pain-reduction therapies.

Several human disease states are candidates for ET_A receptor antagonist pain reduction therapies. Diseases implicated include diabetic neuropathies, trigeminal neuralgia, crushed nerve syndrome, chronic arthritis, and many types of cancer pain (bone, oral, prostate, and breast). In addition to these diseases, several others have been implicated as ET-1 receptor antagonist clinical trial candidates on the basis of their overproduction of ET-1. Research on ET-1's involvement in several painful conditions such as sickle cell crisis, acute chest syndrome, complex regional pain syndrome, and Raynaud's syndrome are currently being investigated, with

Table 3 Participation of ET_A and ET_B receptors in different models

| Model | ET _A receptor | ET _B receptor | References |
|---|---|----------------------------|------------|
| Exogenous application of ET-1 | | | |
| Spontaneous hind paw flinching | Pronociceptive | Antinociceptive | 50,59,61 |
| Abdominal constriction | Pronociceptive | Not tested | 47,48,114 |
| Sciatic nerve application | Pronociceptive | Little response | 55 |
| Nerve recordings | Pronociceptive | Antinociceptive | 50 |
| Spontaneous licking | Pronociceptive | Not involved | 58,60 |
| Mechanical hyperalgesia | Not involved | Pronociceptive | 46,65,68 |
| | Pronociceptive | Pronociceptive | 67 |
| Thermal hyperalgesia | Pronociceptive | Not involved | 60 |
| ET-1 + formalin | Phase I: mediates | Phase I: not involved | 52 |
| | Phase II: mediates | Phase II: mediates | |
| ET-1 + capsaicin licking | Pronociceptive | Not involved | 75 |
| Enhanced carrageenan paw elevation | Pronociceptive | Pronociceptive | 92 |
| No exogenous ET-1 application | | | |
| Postsurgical mechanical hyperalgesia | Pronociceptive | Not tested | 77 |
| Phenylbenzoquinone-induced abdominal constriction | Not involved | Pronociceptive | 146 |
| Pruritus | Not involved | Pronociceptive | 146 |
| Inflammatory pain: thermal hyperalgesia | Pronociceptive | Not involved | 67 |
| Inflammatory pain: mechanical hyperalgesia | Pronociceptive | Pronociceptive | 67 |
| Primed inflammatory pain: paw elevation | Not involved | Pronociceptive | 91 |
| Cancer | | | |
| Sarcoma virus infected | Early and late thermal hyperalgesia | Early thermal hyperalgesia | 105 |
| Sarcoma cells | Mediates nociception | – | 147 |
| Bone cancer | Nociception | Analgesia | 102 |
| Oral cancer | Increase in nociception and mechanical hyperalgesia via ET _A | – | 103 |
| Skin cancer | Pronociceptive; mechanical allodynia | – | 104 |
| Oral cancer melanoma | Pronociceptive in oral cancer model | | 100 |

Abbreviation: ET, endothelin-1.

pain-relieving results being reported. For example, three patients with secondary Raynaud's phenomenon received the mixed ET_A/ET_B receptor antagonist bosentan twice daily for 4 weeks, followed by an increased dose twice daily for 12 weeks. Patient questionnaires using the visual analog scale revealed that pain severity decreased significantly in all patients during the treatment period.¹⁴² ET-1 was investigated as a potential marker for patients admitted to the emergency room for a sickle cell pain crisis and in patients with complex regional pain syndrome.^{143,144}

At this time, the only licensed ET receptor antagonist medications available in the United States and Europe are bosentan, a dual ET_A/ET_B receptor antagonist, and ambrisentan, a selective ET_A receptor antagonist, for the treatment of pulmonary hypertension.¹⁴⁵ The ET_A receptor antagonists or combined ET_A/ET_B receptor antagonists are currently in clinical trials for the treatment of diseases, including prostate, kidney, ovarian, fallopian, and peritoneal cancer, as well as diabetic neuropathy, diastolic heart failure, pulmonary arterial hypertension, pulmonary fibrosis, scleroderma, and subarachnoid hemorrhage.¹⁴⁵ Many of the clinical trials are not aimed at pain relief but are targeting other actions of ET-1 in other systems. However, to gain the maximum benefit from these trials, the pain-producing (or pain-relieving) effects of ET-1 need to be better understood and evaluated. Pain reduction has already been reported in trials of bone and prostate cancer pain.⁴

Therefore, it is imperative that the current trials index changes in pain to elucidate all the possible benefits of endothelin receptor antagonists. This review of the literature on ET-1 beckons to clinical researchers to include a pain analysis within the ongoing studies. Possible pain-relieving adverse effects may be completely overlooked without the needed attention from clinical studies. Significant pain relief may be an additional benefit in patients taking endothelin receptor antagonists for a variety of pathologies.

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Disclosure

The authors report no conflicts of interest in this work.

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