Abstract
Growing evidence implicates the renin–angiotensin system (RAS) in multiple facets of neuropathic pain (NP). This narrative review focuses primarily on the major bioactive RAS peptide, Angiotensin II (Ang II), and its receptors, namely type 1 (AT1R) and type 2 (AT2R). Both receptors are involved in the development of NP and represent potential therapeutic targets. We first discuss the potential role of Ang II receptors in modulation of NP in the central nervous system. Ang II receptor expression is widespread in circuits associated with the perception and modulation of pain, but more studies are required to fully characterize receptor distribution, downstream signaling, and therapeutic potential of targeting the central nervous system RAS in NP. We then describe the peripheral neuronal and nonneuronal distribution of the RAS, and its contribution to NP. Other RAS modulators (such as Ang (1-7)) are briefly reviewed as well. AT1R antagonists are analgesic across different pain models, including NP. Several studies show neuronal protection and outgrowth downstream of AT2R activation, which may lead to the use of AT2R agonists in NP. However, blockade of AT2R results in analgesia. Furthermore, expression of the RAS in the immune system and a growing appreciation of neuroimmune crosstalk in NP add another layer of complexity and therapeutic potential of targeting this pathway. A growing number of human studies also hint at the analgesic potential of targeting Ang II signaling. Altogether, Ang II receptor signaling represents a promising, far-reaching, and novel strategy to treat NP.

Keywords: Angiotensin, Neuropathy, ACE inhibitor, Neuroimmune interactions, Macrophages, Type 1 angiotensin receptor, Type 2 angiotensin receptor, Mas receptor

1. Introduction
Neuropathic pain (NP) is among the most challenging conditions to treat effectively, affecting 7% to 10% of those with chronic pain. The most common NP conditions include diabetic, alcohol, and drug-induced neuropathy, and too commonly proper analgesia is not achieved by currently available medications. As such, many alternative drug targets have been studied for their analgesic efficacy. Angiotensin receptor blockers are one such group of potential targets, and there are a growing number of promising preclinical and clinical studies showing the effectiveness of different Ang II receptor modulators in NP. A large number of studies have explored the pathophysiological roles of the renin–angiotensin system (RAS) pertaining to the vasculature, heart, kidney, and brain, in the contexts of inflammation and damage repair, metabolic dysfunction, and aging. These topics have recently been reviewed comprehensively elsewhere. The present review focuses on RAS signaling in NP and the novel therapeutic opportunities represented by recent discoveries.

The RAS was first described in the late 19th century as an endocrine modulator of systemic blood pressure. Angiotensin II (Ang II) is the major bioactive octapeptide responsible for vasoconstriction and stimulating secretion of aldosterone and vasopressin, increasing sodium and fluid retention. Ang II is derived from the inactive precursor angiotensinogen, which was first shown to be released into the circulation by the liver (Fig. 1). Angiotensinogen is first cleaved by renin, yielding the inactive intermediate angiotensin I (Ang I). Angiotensin-converting enzyme (ACE) then catalyzes the conversion of Ang I into Ang II. Additional proteases that catalyze the production of Ang II were discovered more recently, such as cathepsins, carboxypeptidases, and aminopeptidases. Ang II binds with similar affinity to the G-protein-coupled receptor subtypes AT1R and AT2R in multiple cell and tissue types (Fig. 2). Unlike humans, mice and rats underwent a gene duplication event and express 2 pharmacologically identical isoforms of AT1R with different expression patterns, termed AT1a and AT1b. Other fragments besides Ang II (Ang 1-8) are formed by alternative pathways, such as Ang III (Ang 2-8), Ang IV (Ang 3-8), and Ang (1-7).
These endogenous products activate different receptors and play different physiological roles. Currently, there are 4 separate receptor types described: AT1R, AT2R, AT4R (receptor of Ang IV), and the AT7R/Mas receptor (receptor of Ang (1-7)) (Fig. 1). AT1R preferentially binds Ang II and Ang III. AT2R preferentially binds Ang II and Ang III with similarly high affinity and is a lower-affinity receptor for Ang IV and Ang (1-7). Ang IV also binds to AT4R, also known as insulin-regulated membrane aminopeptidase/IRAP. Ang IV inhibits IRAP, thereby extending the half-life of "analgesia-promoting" peptides that are otherwise cleaved by the receptor’s enzymatic activity (eg, vasopressin, oxytocin, and somatostatin). Ang (1-7) is formed from Ang I and Ang II by the activity of the ACE homolog angiotensin-converting enzyme-2 (ACE2), in an alternate RAS pathway to the ACE/Ang II pathway. Ang (1-7) binds to the Mas1 proto-oncogene GPCR, commonly referred to as the Mas receptor, MasR. This collection of ligands, receptors, and physiological functions has many important implications for NP because they operate in the central and peripheral nervous systems, as well as nonneuronal cells known to influence NP (eg, leukocytes). The modulators of these receptors reviewed in the present article are listed in Table 1.

2. Angiotensin receptors in the brain: relevance to neuropathic pain

2.1. Angiotensin signaling in the brain: angiotensin receptors and functions

AT1Rs at sites controlling autonomic and hormonal responses in the brain are the chief drivers of the classic Ang II-induced pressor and dipsogenic responses. Ang (1-7) is formed from Ang I and Ang II by the activity of the ACE homolog angiotensin-converting enzyme (ACE2), in an alternate RAS pathway to the ACE/Ang II pathway. Ang (1-7) binds to the Mas1 proto-oncogene GPCR, commonly referred to as the Mas receptor, MasR. This collection of ligands, receptors, and physiological functions has many important implications for NP because they operate in the central and peripheral nervous systems, as well as nonneuronal cells known to influence NP (eg, leukocytes). The modulators of these receptors reviewed in the present article are listed in Table 1.
The RAS, and in particular the cerebral RAS, has been implicated in learning and memory, regulation of affective responses, and processing of pain. Local administration of Ang II into the CeA of rats results in hyperalgesia in the tail-flick test. This effect of Ang II on the ventrolateral PAG was inhibited by prior local administration of saralasin, a nonselective AT1R antagonist. The role of the cerebral RAS in hyperalgesia was investigated using Kandalam and Clark’s antagonists and agonists of AT1R and AT2R. Enalapril, Losartan, and Telmisartan were used as AT1R antagonists, while Losartan and Telmisartan were used as AT2R antagonists. The combination of saralasin and losartan showed a synergistic effect in reducing hyperalgesia. Additionally, the use of AT1R agonists, such as losartan and losartan, was found to be effective in reducing hyperalgesia.

2.2. Processes induced by the dysregulation of the cerebral renin–angiotensin system

The diverse functions of the cerebral RAS are still not fully understood. A more complete understanding of the glial and neuronal subtypes involved, the circuitry, and circumstances triggering such signaling events will be required to delineate the functions of its different receptors and neuropeptides in different pathological processes, including NP. Nevertheless, the cerebral RAS seems to play an important role in neuroprotection. Its dysregulation seems to be an important factor in different neurodegenerative diseases, which hints at potential involvement in NP. The role of the cerebral RAS specifically in neurodegenerative diseases has recently been reviewed elsewhere.

2.3. Possible future therapeutic implications of the brain renin–angiotensin system in neuropathic pain

There is evidence to suggest that the cerebral RAS can promote or inhibit pain responses, depending on the cells/structures targeted and the receptor subtypes involved. For example, local administration of Ang II in the caudal ventrolateral medulla caused hyperalgesia in rodent tail-flick and formalin tests. This hyperalgesia was significantly attenuated by local administration of losartan (AT1R antagonist) and saralasin (nonselective AT1R antagonist). These results suggest that Ang II may exert pronociceptive effects in components of the supraspinal pain control system. However, Parlo et al. showed that Ang I, II, or III injected into rat periaqueductual gray induced dose-dependent antinociception in the tail-flick test. This effect of Ang II on the ventrolateral PAG was inhibited by prior local administration of saralasin, a nonselective angiotensin receptor antagonist. Ang II also activates spinally projecting paraventricular nucleus (PVN) neurons by AT1R-mediated attenuation of GABAergic (but not glutamatergic) synaptic inputs into the PVN. This observation is consistent with the finding that electrical stimulation of the PVN increases pain threshold. Because the activity of the ACE2-Ang (1-7)-AT7R/Mas receptor axis tends to oppose the activity of AT1R signaling, augmenting MasR signaling may offer an additional approach to achieve similar analgesia.
In addition to AT1R, cerebral AT2R might also be an important regulator of pain threshold, as indicated by Sakagawa et al. They found increased pain sensitivity in tail-flick and tail-pinching tests in AT2R-deficient animals, a finding that was associated with reduced expression of B-endorphin in the arcuate nucleus of the medial basal hypothalamus in AT2R knockout mice.

In contrast to signaling through AT1R, the activation of AT2Rs exerts mainly neuroprotective effects. Recently, Bhut et al. showed that AT2R activation elicits neuroprotection by inhibiting proinflammatory processes in microglia; AT2R-mediated activation of protein phosphatase 2A prevented Ang II-induced activation of protein kinase C, phosphorylation of p47phox, and proinflammatory activation of microglia. ROS production, proinflammatory microglial activation, and sickness behaviors were also inhibited by AT2R activation in a mouse model of neuroinflammation. Ang IV—AT4R/IRAP signaling is also thought to be broadly neuroprotective. Ang IV increases neuronal glucose uptake through inhibition of GLUT4 vesicular trafficking and inhibits proteolysis of neuropeptides associated with analgesia (eg, vasopressin, oxytocin, and somatostatin). The activation of AT4Rs might offer novel therapeutic opportunities for NP, although more studies are needed to explore these complex effects.

The activation of ACE2 might offer beneficial therapeutic effects by enhancing Ang (1-7) availability. Ang (1-7) mainly exerts trophic effects. Although the exact role of different Ang II activators in NP is a question for the future, the possible therapeutic role of ACE2 activators in NP is a question for the future.

How the highly complex system of the brain RAS influences the development of different pain types, and how these processes might be used therapeutically is far from fully understood. As the neuroprotective effects and inhibitory facilitation of some angiotensin receptor agonists came to light, research began to focus on potential therapeutic implications of the peripheral RAS and angiotensin receptors. Indeed, EMA401 (the first AT2R antagonist analogistic reaching clinical trials) was not found to penetrate the CNS, yet proved to be a potent analogistic for the treatment of NP5,121 (see section 4).

3. Angiotensin receptors in spinal and peripheral neurons and relations to neuropathic pain

3.1. Angiotensin signaling at the spinal cord and dorsal root ganglion

Although the majority of the research on RAS in NP focuses on peripheral injury, there are findings that are likely to be relevant to injury due to CNS trauma, eg, spinal cord injury. Such trauma is characterized by neuroinflammation, leukocyte infiltration, and neuronal apoptosis. The ability of Ang II to induce chemokine expression in myocardium and vascular smooth muscle suggests that Ang II production might also underlie leukocyte infiltration and subsequent pathology after CNS injury. Indeed, Fuchtbauer et al. found that Ang II signaling to astrocytes through AT1R regulates leukocyte infiltration into the CNS in response to axonal lesioning. Blocking AT1R with candesartan significantly increased numbers of infiltrating macrophages. The infiltration of macrophages at sites of neuronal injury is seen across multiple models of neuropathy and shows a strong connection with activation of the RAS, as described in section 4.

Wu et al. hypothesized that cerebral AT2Rs are neuroprotective and support neuronal survival in response to ischemia-induced neuronal injury through inhibiting AT1R. A recent study by Abdul-Muneer addressed neuroinflammation and apoptosis in a cell culture model of neuronal stretch injury. They showed that stretch injury increases Ang II, causing the release of proinflammatory cytokines and oxidative stress. The AT1R antagonist losartan attenuated the oxidative stress, reducing neuroinflammatory and cell death. AT1R expression is upregulated after injury in this model, which was also counteracted by losartan. These interesting findings help further our understanding of the mechanisms by which AT1R antagonists exert anti-inflammatory and neuroprotective effects, but it should be further explored in vivo across different pain models. Notably, some studies suggest that the beneficial (anti-inflammatory and neuroprotective) effects of competitive AT1R antagonists might be due to facilitation of AT2R signaling. This issue is discussed further in section 3.2.

Numerous studies indicate that the spinal cord expresses RAS peptides and receptors described in the cerebral RAS in the previous section. Ang II acts as a neuromodulator in spinal pain transmission, which is supported by the finding that spinal administration of Ang II elicits nociceptive behaviors in different pain models. Ang II and AT2R were shown in peripheral neuronal tissues that are crucial in pain transmission: rat and human dorsal root ganglion (DRG) and in trigeminal ganglia. Transcripts for renin, angiotensinogen, and ACE are all expressed in rat DRG neurons and other cell types (eg, blood vessels and satellite glia). In addition, Ang II colocalized with substance P and calcitonin gene-related peptide (CGRP)-containing DRG neurons, which further supports its role in nociception.

Angiotensinogen and ACE mRNA were detected in rat DRG by Patil et al. Furthermore, a combination of in situ hybridization and immunohistochemistry revealed colocalization of angiotensinogen mRNA and Ang II immunoreactivity in most rat DRG neurons. This indicates angiotensinogen can be produced intraneuronally in the DRG and that uptake from circulation may not be necessary for the local formation of Ang II. Neuronal expression in DRG is in contrast to the brain, where angiotensinogen is predominantly expressed by astrocytes, neurons, and cerebral endothelial cells to a lesser extent.

Mitogen-activated protein kinase (MAPK) phosphorylation seems to be a key player in Ang II-mediated transduction of pain signals. Mitogen-activated protein kinases are activated by phosphorylation and play crucial roles in cell signaling and gene expression. Evidence shows that spinal p38 MAPK is activated after neuronal injury; consequently, it is a major contributor to the development and maintenance of NP. Several Ang II signaling modulators influence pain transmission, at least in part, by counteracting Ang II-induced MAPK signaling. Another key component of Ang II-mediated signaling is p42/p44 MAPK (ERK1/2), which is essential for induction of AT2R-mediated neurite outgrowth. It is conceivable that such Ang II-mediated neurite outgrowth/regeneration is beneficial when target reinnervation is achievable after nerve injury. However, futile regeneration is maintained if the nerve injury is too severe to be repaired. Taken together, AT2R activation induces pleiotropic effects, both of which have potential clinical significance. p38 MAPK activation contributes to NP symptoms, whereas the ERK1/2 pathway seems to moderate the neurotrophic effects. Although the exact role of different Ang II receptors (mainly AT1R vs AT2R) in the development and possible management of NP is not yet clear, the following
sections describe studies that have evaluated the relationships between different angiotensin receptor subtypes and pain.

3.2. Angiotensin II type 1 receptor modulators and neuropathic pain

Considering that AT1R signaling is proinflammatory in other tissues and organ systems, it is expressed along the pain neuraxis, it was hypothesized that AT1R also contributes to pathological pain states (the reviewed AT1R modulators are listed in Table 2). For example, Nemoto et al. found that intrathecal losartan (an AT1R antagonist) produces an antinociceptive effect through the inhibition of p38 MAPK phosphorylation in the mouse formalin test. Subsequent studies in NP models showed that AT1Rs were found to be expressed in both neurons and satellite cells in the DRG. Affirming these results, Yuksel et al. found that systemic delivery of telmisartan (also an AT1R antagonist) significantly improved nerve healing by inhibiting production of IL-1β and caspase-3 in nerve crush and transection NP models in rats. Based on these findings, the authors concluded that AT1R inhibitors could be used to improve peripheral nerve regeneration after injury.

Diabetic neuropathy represents a unique intersection of the “classic” hemodynamic effects of Ang II signaling and its more recently described role as an inflammatory mediator. Increased circulating Ang II in diabetes is known to contribute to renal injury, which can progress to chronic kidney disease and uremic neuropathy. It is also well established that vascular dysfunction in diabetic neuropathy drives nerve injury by impairing endoneurial blood flow. This leads to energetic dysfunction, inflammation, and cell death. In one study, streptozotocin-induced diabetic rats were treated for 12 weeks with enalapril, an ACE inhibitor, or L-158809, an AT1R antagonist. Interestingly, treatment of diabetic rats with either compound reduced superoxide levels in the aorta, prevented the diabetes-induced impairment of vascular relaxation, and improved acetylcholine-mediated vasodilation. Similarly, Ogata et al. showed that neuropathic symptoms of streptozotocin-induced diabetic mice were alleviated by intrathecal administration of losartan, an AT1R antagonist, but not by PD123319, an AT2R antagonist. It seems that expression of spinal ACE is increased under diabetic conditions. This leads to an increased level of Ang II, accompanied by the AT1R-dependent phosphorylation of p38 MAPK. Collectively, this supports the observations that ACE inhibitors and/or Ang II receptor blockers may be effective treatments for diabetes and the attendant vascular and neural dysfunction 

(Tables 2 and 3). However, mechanistically disentangling direct effects of Ang II on neuronal excitability from effects secondary to cardiovascular/renal dysfunction may prove challenging.

Studies using inflammatory pain models suggest that AT1R antagonists may not be universally analgesic or may differ in their effectiveness between inflammatory and NP models. Costa et al. showed that carrageenan-evoked inflammatory pain was augmented by the AT1R antagonist losartan, as well as the AT7R/Mas receptor antagonist A779. These antagonists showed no effect in chronic constriction injury (CCI). This serves as a clear indication that suitability of analgesics must be tested across different pain models and animal strains. Clearly, the type of pain-inciting insult highly influences, or even determines, the effectiveness of different therapeutics.

A recent study by the Snyder laboratory showed a reciprocal interaction of AT1R with the ion channel transient receptor potential vanilloid 4 (TRPV4) in choroid plexus epithelia. The results indicated that TRPV4 activation and Ca2+ influx inhibits AT1R signaling. These results also hint at the possibility of AT1R-mediated inhibition of TRPV4. Such reciprocal regulation is likely conserved across other GPCR–TRP channel pairs, building upon an earlier hypothesis that Ang II receptors expand their functional diversity through dimerization with other GPCRs. Because there is an apparent role of TRPV4 in NP, the implications for NP should be further investigated.

3.3. Angiotensin II type 2 receptor modulators and neuropathic pain

Anand et al. showed that expression of AT2R in small-/medium-diameter human and rat DRG is partly colocalized with TRPV1 expression. A study by the Benitez laboratory also showed that AT2R is preferentially expressed in nonpeptidergic (IB4-binding) likely C-nociceptors and it is also present in peptidergic (trkA+/SP+) neurons that encompass both small C-nociceptors and medium-size A-neurons, which are crucial in pain transmission. In cultured DRG neurons, Ang II potentiated Ca2+ flux evoked by the

Table 2
Effects of AT1R antagonists.

| Compound                  | Model                                      | Outcome                                                                 | Citation |
|---------------------------|--------------------------------------------|------------------------------------------------------------------------|----------|
| Losartan                  | CVM injection of Ang II (rat)               | Reduced hyperalgesia (tail-flick, formalin tests)                      | 79       |
|                           | Formalin injection (mouse)                 | Antinociceptive (through p38 MAPK inhibition)                          | 93       |
|                           | In vitro neuronal stretch injury (rat)      | Attenuated oxidative stress, neuroinflammation, cell death             | 1        |
|                           | Ang II (i.t.; mouse)                        | Antinociceptive (delivered i.t.)                                      | 95       |
|                           | Streptozotocin-induced diabetic neuropathy (mouse) | No effect on capsaicin responses                                      | 4        |
|                           | Cultured human, rat DRG neurons             | Alleviated mechanical hyperalgesia                                     | 66       |
|                           | Paclitaxel-induced neuropathy (rat)         | No effect in Randall–Selitto test                                     | 24       |
|                           | Chronic constriction injury (rat)           | No effect on von Frey sensitivity                                     | 117      |
|                           | SNI (mouse)                                | No effect on nerve regeneration                                       | 106      |
|                           | Sciatic nerve crush (rat)                   | Reduced Ang II-induced IL-6 secretion                                 | 83       |
|                           | Astrocyte culture (rat)                     |                                                                         |          |
| Candesartan               | Vincristine-induced neuropathy (mouse)      | Restored normal tactile sensitivity                                    | 12       |
|                           | LPS-induced neuroinflammation (rat)         | Alleviated neuroinflammation                                           | 14       |
| Telmisartan               | SNI (rat; axonotomy vs anastomosis)         | Improved nerve regeneration                                           | 141      |
|                           | Streptozotocin-induced diabetic neuropathy (rat) | Prevented vascular abnormalities                                     | 22       |

CVM, caudal ventrolateral medulla; dorsal root ganglion; MAPK, mitogen-activated protein kinase; SNI, spared nerve injury.
TRPV1 agonist capsaicin, along with increasing cAMP levels and promotion of neurite outgrowth. Treatment with EMA401, a selective AT2R antagonist, inhibited capsaicin-evoked responses (compounds acting on AT2R are listed in Table 4). Although human limb peripheral nerve segments proximal to injury showed reduced AT2R, expression levels were preserved in painful neuromas. This suggests maintained expression in regenerating nerve fibers. By contrast, AT1R immunoreactivity was absent from neurons, but strongly positive in vascular structures, human DRG, and in other tissues. This is in agreement with other studies, which have indicated that the expression pattern of AT2R may contribute to its viability as a target for the treatment of NP. Accordingly, the AT1R antagonist losartan had no effect on DRG neuron responses to capsaicin. The possible therapeutic role of AT2R is also underlined by the finding that an inflammatory soup (histamine, ATP, PGE2, bradykinin, serotonin, IL-6, TNFα, and substance P) was found to upregulate AT2R but not AT1R mRNA levels in cultured rat DRG neurons. Indeed, Smith et al. showed that increased Ang II/AT2R signaling in the DRG of CCI rats is attenuated by the AT2R antagonist EMA300, with similar attenuation in a bone cancer pain model. In CCI mice, the analgesic effect was abolished in AT2R knockouts and had an intermediate effect in the hemizygotes, affirming a role for AT2R. AT2R blockade by EMA300 resulted in the inhibition of p38 MAPK and p44/p42 MAPK activation in the rat DRG. These results also corroborated the colocalization of Ang II with the small-/medium-diameter nociceptive neuronal markers, substance P, and CGRP in rat and human tissues. Further supporting the possible future therapeutic use of AT2R antagonists, different compounds produced analgesic effects in several different animal models of NP, including the following: spared nerve injury-induced mechanical hypersensitivity in mice (PD123319/EMA200;), a rat model of antiretroviral toxic polyneuropathy (EMA200, EMA300;), and the CCI model in rats (EMA200, EMA300, and EMA400;). Our recent findings also underline the promising effects of AT2R antagonists in NP. Mechanical and cold pain hypersensitivity (assessed using reflexive and nonreflexive measures of pain sensitivity) were measured in spared nerve injury mice after systemic administration of PD123319/EMA200. Despite observing analgesic efficacy, AT2R expression was not detected in DRG neurons by RNA sequencing. AT2R-GFP reporter mice were also negative in DRG histology. These apparently conflicting data could indicate that the injury model, duration, and species used can influence AT2R expression, and that cell types other than DRG neurons may also be targets of AT2R antagonists. This is discussed further in section 4. Nonetheless, these results independently verified that the analgesic action of AT2R antagonism is conserved across the different subtypes of EMA compounds used.

Although blockade of AT2R signaling seems to be analgesic, it seems that AT2R agonists are neuroprotective and/or neurotrophic. More than 2 decades ago, Lucius et al. found that AT2R promotes the axonal elongation of postnatal rat retinal explants and DRG neurons in vitro, and axonal regeneration of retinal ganglion cells after optic nerve crush. Cotreatment with the AT2R antagonist PD123317 (but not the AT1R antagonist losartan) abolished the Ang II-induced axonal regeneration. In a more recent study, Ang II was administered through osmotic minipump to injured sciatic nerve in rats. The authors found that Ang II treatment promoted functional recovery, which was fully inhibited by AT2R antagonist PD123319. Once again, the AT1R antagonist losartan had no effect. This was the first study to present direct evidence for an involvement of the AT2R in peripheral nerve regeneration. There are now several publications.
highlighting the role of the RAS in neuronal development and protection.26,31,68 For example, Chakraborty et al.20 showed that locally synthesized Ang II in rat DRG plays a role in estrogen-mediated sprouting. Estrogen-induced neurite outgrowth was inhibited by the ACE inhibitor enalapril. 17β-Estradiol was also found to upregulate AT2R expression, and the neurotrophic action of estradiol was counteracted by the AT2R antagonist PD123319. Based on the neurotrophic role of AT2Rs, it is hypothesized that AT2R activation might be able to improve NP by facilitating neuronal protection or regeneration. In a recent study, Bessaguet et al.13 analyzed the effects of PD123319/EMA200 (AT2R antagonist) in resiniferatoxin-induced sensory small-fiber neuropathy in mice. They showed that AT1R blockade by the competitive antagonist candesartan prevented functional sensory neuropathy. This effect was not ascribed to AT1R inhibition per se, but to the resultant increase in Ang II binding to AT2R. This conclusion is based on the finding that AT2R blockade does not prevent resiniferatoxin-induced neuropathy, and the effect of candesartan could be blocked by PD123319/EMA200 in wild type but not AT2R-deficient mice. Therefore, the beneficial effects of candesartan could result from blunting of AT1R-mediated negative feedback on the release of renin, thereby increasing Ang II-AT2R signaling and enhancing neuroprotection. In a murine model of vincristine-induced mechanical allodynia, both candesartan and the AT2R-selective agonist Compound 21 completely restored normal tactile sensitivity. However, only Compound 21 displayed neuroprotective effects against vincristine-induced neuronal damage. This study provides further evidence of AT2R agonist-induced neuroprotection.12 A study by Bhat et al.15 similarly connected the beneficial effects of candesartan to facilitation of AT2R signaling. Candesartan treatment alleviated neuroinflammation in rats and in astroglial and microglial cells. Such effects were associated with elevation of AT2R expression and blocked by AT2R antagonists.

Marion et al.78 described a novel mechanism involving AT2Rs, in which a mycolactone toxin, Mycobacterium ulcerans, exerts analgesia. Mycolactone-induced AT2R signaling elicited Gq signaling, triggering a cascade culminating in PGE2 synthesis and leading to the opening of TRAAK channels, hyperpolarization, and analgesia. These results should be explored further using other well-established AT2R agonists (eg, Compound 21) under NP or other pain conditions to better understand mechanism(s) underly the AT2R-induced analgesia and the ability of PGE2 to participate in analgesia and hyperalgesia in different pathologies.72 These findings are intriguing because several clinical and preclinical studies suggest the utility of AT2R antagonism for NP. It is possible that the analgesic effects of AT2R depend on the nature of the pain-inducing insult or the cell type(s) expressing AT2R. Perhaps AT2R antagonists inhibit a Gαq pathway in nociceptors, which converges with the nerve growth factor-TRPV1 pathway. By contrast, pathways activated by compounds such as mycolactone perhaps predominate in leukocytes and/or the CNS.27 Of note, our study indicates the involvement of macrophage AT2Rs and neuronal TRPA1 channels in the analgesic actions of AT2R antagonists, as described in section 4.

In summary, AT2R agonists have been found to be neuroprotective because neuronal regenerative processes are commonly hampered under neuropathic conditions. However, there are numerous studies showing the effectiveness of AT2R antagonists, as they seem to alleviate NP symptoms. Further studies are needed to investigate these somewhat competing therapeutic possibilities, and the extent to which these neurotrophic and analgesic properties of AT2R (or AT1R) might be used clinically in NP. For example, careful optimization of dosing, delivery, and NP stratification may be required to offset nerve regeneration against analgesia. Based on our current knowledge, AT2R antagonists are among the most promising novel compounds for the future management of NP.106

3.4. Other angiotensin receptors, modulators, and neuropathic pain

Ang (1-7) is mainly produced by ACE2 from Ang II and binds to the G-protein-coupled AT7R/Mas receptor, reducing inflammation, fibrosis, and insulin resistance.119 Ang (1-7) and AT7R/Mas activation seems to counteract the harmful effects of Ang II-mediated signaling (pronociception and proinflammation) by inhibition of MAPK activation25 (Mas receptor modulators are listed in Table 5). In a recent study, Yamagata et al.138 postulated that Ang (1-7) production is downregulated in leptin-deficient ob/ob diabetic mice, which is accompanied by a loss of ACE2-positive neurons. Intrathecal administration of Ang (1-7) in ob/ob mice attenuated hyperalgesia. This effect was counteracted by A779, a Mas receptor antagonist. SB203580, a p38 MAPK inhibitor, also attenuated hyperalgesia, and Ang (1-7) inhibited the phosphorylation of spinal p38 MAPK. This study again underlines the important role of RAS-mediated p38 MAPK phosphorylation in pain transmission and the beneficial counteracting role of the ACE2/Ang (1-7)/Mas receptor pathway. Ang (1-7) signaling has also been linked to the NO/cyclic GMP pathway. NO is capable of activating ATP-sensitive K+ channels (KATP) through increased intracellular cGMP, which has been implicated as an antinoceptive mechanism for several different drugs.16 Costa et al.23 investigated this pathway in relation to Ang (1-7). They showed that Ang (1-7) induced NO release and demonstrated an antinoceptive effect in rats after hind paw injection of PGE2. This antinoceptive was antagonized by the nonselective NO synthase inhibitor L-NOarg, the selective neuronal NO synthase inhibitor L-NPA, and the KATP channel blocker glibenclamide. Although these findings indicate that Ang (1-7) activates the NO/cyclic GMP/KATP channel pathway, a role for Ang (1-7) in the treatment of NP should be further evaluated.

| Table 5 |
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| Agents acting on MasR. |
| **Compound** | **Model** | **Outcome** | **Citation** |
| Agonists | | |
| Ang (1-7) (I.p.) | Metastatic bone pain (mouse) | Attenuated spontaneous, evoked pain | 41 |
| Ang (1-7) (I.t.) | Ang II (I.t.)-induced nociception (mouse) | Attenuated nociceptive behavior | 91 |
| | Obese diabetic (ob/ob) mouse | | 138 |
| Antagonists | A779 | CCI (rat) | No effect | 24 |

CCI, chronic constriction injury.
ACE2 is of tremendous growing interest after the discovery that it is the main cell entry receptor for the novel coronavirus SARS-CoV-2. The spike protein of SARS-CoV-2 binds ACE2. Given the expression of ACE2 in the peripheral and CNS and several reports of neurological complications of COVID-19, pain is a raising concern as a potential complication of this novel highly contagious infection. The dysregulation of ACE2/Ang (1-7)/Mas receptor pathway by the virus-induced damage. A study by Okamura et al. showed that monocytes/macrophages promote the development of NP symptoms by fostering neuronal angiogenesis by RAS antagonism. Migrating leukocytes are crucial in the development of different pain states. However, the circumstantial findings shed light on the possible intriguing neuroimmune mediated by AT1R activation.

4. Effect of the renin–angiotensin system on immune system function and implications for neuropathic pain

Ang II and AT2R have been shown to be expressed by sensory neurons and by nonneuronal cells. The density of angiotensin receptors is developmentally regulated. Although AT1R is expressed in most adult tissues including the heart, blood vessels, brain, and kidney, AT2R expression was (until recently) thought to be largely restricted to embryonic, fetal, and neonatal tissues. Immune cells are now considered to be crucial in the development of pain, and the infiltration of various immune cells into peripheral nervous structures and their profound influence on pain development is a well-known phenomenon. Circulating mononuclear cells, macrophages, and T-lymphocytes are known to express ACE. Elevated ACE expression is associated with enhanced myeloid cell activity, although the implications of this for NP have not been explored. Nevertheless, these findings shed light on the possible intriguing immune interaction in the complex mechanism of pain transmission and the development of different pain states. However, the circumstances that trigger immune system RAS contribution to NP, and/or the changes in leukocyte trafficking and inflammatory phenotype that may result from RAS signaling require further study.

A study by Nataraj et al. was one of the first to describe the capability of Ang II to influence immune responses; the immunosuppressive effects of calcineurin inhibitors were potentiated by RAS antagonism. Migrating leukocytes are crucial in lesion development, in which Ang II is an essential mediator. In addition, macrophages express all components of the RAS. These cells infiltrate local tissues after neuronal injury, and further promote the development of NP symptoms by fostering neuronal damage. A study by Okamura et al. showed that monocytes/macrophages upregulate RAS expression during differentiation, a phenotypic change that underlies the contribution of macrophages to development of atherosclerosis. Moreover, an in vitro study by Guo et al. showed that Ang II acts directly on macrophages; Ang II contributes to the production and release of proinflammatory cytokines TNF-α and IL-1β in macrophages through AT1R activation.

Several studies show that the RAS is highly involved in immunological responses under pathological conditions (eg, during inflammation). Nabab et al. showed how Ang II induces neutrophil accumulation. In this study, Ang II provoked rapid neutrophil recruitment, mediated through the release of CXC chemokines such as CINC/KC and MIP-2 in rats and IL-8 in humans. This may contribute to neutrophil infiltration in acute myocardial infarction. In a study by Moreno et al., Ang II infusion resulted in the infiltration of inflammatory cells and stimulated cell proliferation. Interestingly, these pathogenic events were attenuated by atorvastatin, which can inhibit Ang II-induced activation of NF-κB and chemokine expression. Although Guo et al. did not investigate NP as a readout, their results highlight the pathophysiological role of macrophage AT1Rs in inflammatory processes. Treating macrophages with Ang II upregulated AT1R expression and increased the production of inflammatory mediators (TNF-α, IL-1β, IL-6, and IL-10) and reactive oxygen species. These effects were blocked by ZD7155, an AT1R antagonist. These results indicate a possible mechanism for the analgesic actions of different AT1R antagonists (see section 3.3) and should be further investigated in relation to NP and other different pain diseases. AT1R antagonists are associated with reduced perineural invasion in head and neck cancer, although it is not yet clear if this is due to an AT1R-mediated effect on chemokine expression. Collectively, these findings are likely to be relevant for NP because leukocyte infiltration and the consequent proinflammatory actions are known to be significant contributors to development and maintenance of NP.

Numerous studies show that the RAS is involved in (auto) immune responses and modulation of T-cell and macrophage function, with Ang II typically thought to drive proinflammatory macrophage responses. Khan et al. investigated the effects of the AT2R antagonist EMA300 on CCI rats. Consistent with their earlier studies, they observed a CCI-induced increase in Ang II expression in DRG. This increase was underpinned at least in part by infiltration of Ang II/AT2R-expressing CD3+ T cells. This increase in Ang II levels was reversed at the time of peak analgesia by administration of a single intraperitoneal dose of EMA300. At the point of peak analgesia, there was also a significant reduction in Ang II/AT2R signaling and its downstream mediators, p38 MAPK and p44/p42 MAPK. These observations are consistent with the hypothesis that cells infiltrating sites of inflammation/tissue injury establish a local RAS. Subsequent AT2R activation then elicits nociceptor hypersensitivity and sprouting. This results in heightened thermal and mechanical sensitivity, as shown in a recent study of CFA-induced inflammation in rats. This study showed that the Ang II-synthesizing proteins renin and angiotensinogen were largely absent from the hind paw after saline injection, but abundant in T cells and macrophages in CFA-injected paws. These findings correlate with an earlier study by Thomas and Hoffman, who discovered decades ago that macrophages express specific receptors for Ang II and related peptides that were thought to be responsible for uptake of Ang II by macrophages.

Our laboratory has previously investigated a possible role of the RAS in neuroimmune interactions in NP conditions. We found that local AT2R activation by Ang II in peripheral macrophages leads to chronic pain hypersensitivity associated with nerve injury/neuropathy. AT2R antagonism provided effective analgesia in neuropathic but not inflammatory pain, although any qualitative difference between acute and more chronic dosing regimens was not explored. In addition, Ang II was elevated in the injured sciatic nerves of mice, and an AT2R antagonist dose-dependently attenuated mechanical hypersensitivity. Our findings suggest that macrophages infiltrating sites of injury induce persistent neuropathic mechanical and cold pain hypersensitivity through the activation of AT2Rs. It is worth noting that macrophages at the site of injury seem to be major contributors to pain hypersensitivity in this model, but macrophage infiltration/proliferation elsewhere along the pain neuraxis (eg, in the DRG) is an area of active study and likely also contributes. Activation of macrophage AT2R induces intercellular crosstalk between peripheral macrophages and sensory neurons, mediated by AT2R-to-TRPA1 redox signaling. Ang II was again shown to induce macrophage infiltration, and AT2R activation macrophages triggered ROS/RNS production, which can
transactivate TRPA1 channels on sensory neurons to induce NP, as has been previously described. The lack of detectable expression of AT2R in mouse and human DRG tissue in these studies indicates that the relative contribution of macrophage-centric mechanisms vs those mechanisms involving other leukocytes and/or direct modulation of DRG neurons (as described in section 3.3) requires further study. Nevertheless, selective AT2R antagonists such as EMA401 and PD123319 have now attenuated NP in humans and in multiple pain model studies conducted by independent research groups. Our study directly identifies immune cell-to-sensory neuron signaling crosstalk, which underlies peripheral nociceptor sensitization. These findings collectively describe a prominent neuroimmune interaction in the development of pain and represent a newly discovered mechanism underlying the analgesic action of AT1R and AT2R antagonists. Ultimately, the emerging ability of RAS modulators to simultaneously target neuronal physiology and pathophysiology, leukocyte infiltration, and inflammation make them a uniquely promising class of analgesic drug candidates.

5. Human studies showing the involvement of the renin–angiotensin system in the development and possible management of pain

Although the majority of data on the role of RAS in pain development originate from preclinical rodent studies, there are a few human studies supporting those findings. As described previously, Ang II and AT2R were shown both in rat and human DRG and in trigeminal ganglia, key points in pain transmission (see section 3.1.). Colocalization of Ang II with the small-/medium-diameter nociceptive neuronal markers, substance P, and CGRP was also demonstrated in human tissues. There are numerous studies involving human tissues suggesting the mechanistic role of Ang II receptors in pain development, which strongly validates the results of the preclinical studies discussed above. One of the early examples of human clinical studies is a study by Drummond, which showed that Ang II administered to healthy volunteers by iontophoresis increased thermal sensitivity, presumably through vasoconstriction and ischaemia. In the late 1990s, Malik et al. investigated the beneficial effects of the ACE inhibitor trandolapril in patients with diabetic NP. In this double-blind, placebo-controlled trial, trandolapril improved neuronal function, assessed by peroneal nerve conduction velocity. These beneficial effects were observed even in normotensive diabetic patients. However, neuropathy symptom and deficit scores showed no improvement. In addition, the sample size was relatively small (41 patients with diabetic NP), and deficit scores showed no improvement. In addition, the sample size was relatively small (41 patients with diabetic NP), and deficit scores showed no improvement. In addition, the sample size was relatively small (41 patients with diabetic NP), and deficit scores showed no improvement. In addition, the sample size was relatively small (41 patients with diabetic NP), and deficit scores showed no improvement. In addition, the sample size was relatively small (41 patients with diabetic NP), and deficit scores showed no improvement. In addition, the sample size was relatively small (41 patients with diabetic NP), and deficit scores showed no improvement. In addition, the sample size was relatively small (41 patients with diabetic NP), and deficit scores showed no improvement. In addition, the sample size was relatively small (41 patients with diabetic NP), and deficit scores showed no improvement.

6. Summary and conclusions

Renin–angiotensin system signaling has expanded far beyond its initial description as an endocrine controller of hemodynamics. Additional enzymes, receptors, peptide fragments, and sites of expression discovered in the past several decades led to the discovery of RAS signaling within the nervous system. It is quickly becoming clear that there are pathophysiological ramifications of angiotensin signaling at all levels of the pain neuraxis, whether in the brain, spinal cord, DRG, or leukocytes infiltrating sites of neurological damage. There remains a lack of consensus

resonates with these observations because they showed the ability of losartan to augment acute pain (section 3.2). There is additional evidence that hemodynamics can influence pain sensitivity, which could partially explain the observed effects of different RAS inhibitors in humans. Some studies have shown the association of hypertension with hypeoalgesia in dental pain. The authors attributed the observed reduction in pain threshold of hypertensives receiving ARBs to AT1R signaling. It is possible that the inhibited degradation of substance P and/or bradykinin underlie these contradictory effects, which have been hypothesized to contribute to complex regional pain syndrome. Ultimately, the systemic/hemodynamic effects and the local/proinflammatory effects may interact and underlie the inconsistencies, but further work is needed to fully exploit targeting of Ang II signaling for pain relief. Nevertheless, acute pain perception in healthy or not neuropathic individuals highly differs from the complex changes observed in NP conditions.

5. Human studies showing the involvement of the renin–angiotensin system in the development and possible management of pain

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Juhi Kalra et al. studied the modulation of pain perception by ramipril and losartan, an ACE inhibitor and AT1R blocker, respectively. The pain perception threshold of 30 healthy individuals was assessed after a single dose of placebo or test compound using a sphygmomanometer-based pressure pain test. Ramipril lowered the measured pain thresholds, and both compounds lowered the maximal tolerated pain 4 hours after administration. The mechanism by which these drugs might influence pain perception would benefit from further study in a larger number of individuals. Notably, a study by Costa et al.
regarding the circumstances under which RAS inhibition would be most beneficial for NP; this may depend to a large extent on the desired outcome (neurotrophic vs analgesic). Clearly, Ang II signaling is a powerful modulator of inflammatory-immunological responses involved in different painful pathological conditions that influence central and peripheral neuronal functions, including NP. A more detailed understanding of the role of RAS in neuroprotection (eg, AT2R initiated neuronal protection) or subsequent activation of different pain pathways (eg, the pain-sensitizing effect of AT2R activation through TRPA1) is likely to offer more opportunities for therapeutic modulation. The current literature supports targeting the RAS to treat NP as a worthwhile endeavor because a preexisting suite of medications indicated for hypertension can be used to guide future therapeutic development. To fully understand the potential role of AT1Rs, AT2Rs, and other components of the RAS for the future management of different pain states, more studies evaluating their impact on multiple clinically relevant pain modalities should be explored.

Disclosures

The authors have no conflicts of interest to declare.

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