

Neuropathic pain: emerging treatments

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Neuropathic pain remains one of the most challenging of all neurological diseases and presents a large unmet need for improved therapies. Many mechanistic details are still lacking, but greater knowledge of overlapping mechanisms and disease comorbidities has highlighted key areas for intervention. These include peripheral and central hyperexcitability. Among the molecular drivers are ion channels (Nav1.7, Nav1.8, Nav1.3, Cav2.2, and alpha2-delta subunits) whose expression is changed during neuropathic pain and their block shows therapeutic utility. Block of a number of ligand-gated channels [transient receptor potential (TRP) V1, TRPM8, and neuronal nicotinic receptors (NNRs)], important in neural sensitization, may also prove beneficial. Other approaches, such as the modulation of peripheral excitability via CB1 receptors, reduction of spinal excitability through block of glutamate receptors (metabotropic glutamate receptor 5 and alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate), block of activated spinal neuroglial (CCR2 and P2X7), or increasing spinal inhibition by enhancing monoaminergic activity, all offer exciting opportunities currently being validated in the clinic. Finally of note is the emergence of biological approaches, for example, antibodies, siRNA, gene therapy, offering powerful therapeutic additions with which to redress the neurological disease imbalances causing neuropathic pain.

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Of all the neurological diseases, neuropathic pain is one of the most challenging with respect to understanding the relationships between symptoms and mechanisms, and rationalizing approaches to treatment.¹² It is not surprising therefore that effective and safe neuropathic pain treatment remains a large unmet therapeutic need.

Most (90%) neuropathic pain states have been considered to arise from peripheral [peripheral nervous system (PNS), post diabetic neuralgia (PDN), post herpetic neuralgia (PHN), post traumatic neuralgia (PTN), and iatrogenic injuries] rather than central nervous system (CNS) injuries [stroke, multiple sclerosis (MS), and Parkinson's disease (PD)], although emerging functional magnetic resonance imaging data have been highlighting that some prevalent chronic pain states [low back pain (LBP), fibromyalgia; and there may be more] may also present with secondary CNS neurodegeneration.^{6 59}

Current chronic neuropathic treatments indicate general insensitivity to non-steroidal anti-inflammatory drugs and relative resistance to opioids, but can be treated by high opioid doses⁵³ at the expense of untoward side-effects. Treatments of choice, or treatments that have received regulatory approvals, include ion channel blocking drugs such as the anticonvulsants gabapentin (Neurontin) and pregabalin (Lyrica).⁸⁵ Overall some 10–30% of pain patients are responsive to these drugs which suffer dose

limitations with respect to efficacy and side-effects (dizziness, sedation, and weight gain). Similarly, antiarrhythmic drugs including mexiletine are claimed to provide pain relief in some 50% of patients but also suffer severe side-effect limitations (sedation tachycardia, hypertension, and weight gain). In some cases, the antidepressant drug amitriptyline, acting as an ion channel and monoamine modulator, has been shown to provide pain relief. Building on this, the serotonin–norepinephrine reuptake inhibitor (SNRI) duloxetine has been approved to treat chronic depression comorbidity and PDN pain,³³ providing a differentiated therapeutic approach that may harness endogenous monoaminergic pathways, addressing important aspects of pain/depression comorbidity.^{33 74 98} Other current therapeutic initiatives are either following these innovative approaches, using clinically validated drugs, or drug combinations, or are providing preclinical and clinical validation for progression of new concepts. For the most part, these new therapeutic approaches are addressing improvements in either efficacy or safety relative to the current clinical choices (summarized in Table 1).

There are many emerging opportunities, arising from the growth of animal and human pathobiology of chronic

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Table 1 Emerging targets and treatments

Drug	Company	Mechanism	Phase	Indications
Prialt (ziconotide)	Elan/Eisai	N-type Ca-channel blocker, intrathecal	Launched	Severe chronic pain
Lacosamide (Vimpat, Harkoseride)	Schwarz Pharma	Nav block	Ph3	Positive in PDN. Evaluation in fibromyalgia and OA ongoing
Ralfinamide	Newron	Nav blocker MAO-inhibitor	Ph2	Evaluation in several NP conditions
Alipamide NW1029	J&J	Glut antag/GABA agonist/Na blocker	Ph3 (PDN)	Launch for epilepsy and migraine. Effective in PDN
Topamax topiramate				
ADX10059, ADX48621	Addex	mGluR5 inhibitor	Ph2	Efficacy in acute migraine. Ph2 planned for NP
AZD 2066	AstraZeneca	mGluR5 inhibitor	Ph1	Ongoing
XP13512	GSK/Xenoport	Pro-gabapentin	Ph 2	Recruiting Ph2
Tezampanel NGX426	TorreyPines	AMPA/kinate antagonist	Ph2	Ongoing
TC6499	GSK/Targacept	Neuronal nicotinic receptor agonist	Ph1	Recruiting Ph1
Retigabine	Valeant	Potassium channel KCNQ2/3 opener	Ph3	Efficacy in epilepsy, PHN
NGD8243 (MRK2295)	Merck/Neurogen	TRPV1 antag	Ph2	Postop dental pain
GRC-6211	Lilly/Glenmark	TRPV1 antag	Ph2	Ongoing for NP and OA
AMG 986	Amgen	TRPV1 antag	Ph1	In progress
AZD1386	AstraZeneca	TRPV1 antag	Ph1	In progress
Transacin (Transdolor, NGX-4010) (topical)	NeurogesX	TRPV1 agonist (capsaicin)	Ph3 (PHN, HIV) and Ph2 (PDN)	Pain reduction in HIV and PHN
Sativex, GW-1000, Buccal spray	GW Pharma	Cannabinoid-receptor agonist	Ph3, Ph2 in PDN	Approved for cancer and MS pain. Effective in NP with allodynia
IP 751, CT-3, ajulemic acid	Indevus	CB1 receptor-agonist/CoX-2/IL-1 block	Ph2	Reduces spinal and nerve injury pain
KDS2000 (topical)	Kadmus Pharma	Cannabinoid	Ph 2	Progress in PHN and PDN
AZD 1940	AstraZeneca	Cannabinoid-receptor agonist	Ph1	Progress
TRO-19622 (oral)	Trophos	Unknown	Ph2a	Recruiting PDN patients
RN624 PF-4383119	Pfizer/Rinat	Anti-NGF mAb	Ph2 (OA)	Recruiting for PHN, LBP, bone pain, cystitis
AMG403	Amgen	Anti-NGF mAb	Ph2 starting	
SB-509	Sangamo	Plasmid DNA to up-regulate VEG-F	Ph2 (PDN)	Disease modifying for diabetes

pain, for targeting key molecular events that underlie pain mechanisms and cellular processes. In this review, I will select some of the most promising targets and provide an assessment of the most advanced concepts that are in their early phases of clinical development.

Pain mechanisms and targets

To date, most drug discovery approaches for neuropathic pain have been based on symptom management, directed at the most commonly described clinical symptoms namely spontaneous pain, mechanical and cold allodynia, hyperalgesia, and hyperpathia. Little attention has been devoted to understanding or addressing the treatment of severe dysesthesias such as numbness, tingling, or prickling that also appear as cardinal features of the neuropathic pain spectrum. Multiple symptoms (and mechanisms) may be present at the same time in neuropathic pain with features that change over time.¹² Indeed, it seems likely that there is an aetiological and progressive relationship between the initiation and maintenance of chronic neuropathic pain providing clinical challenges and treatment opportunities.

With the growth of evidence-based and translational medicine, we are understanding the relationship between

symptoms and key cellular and molecular mechanisms. This provides powerful strategies to direct rational drug therapy rather than follow clinical serendipity. The major cellular mechanisms include ectopic or spontaneous nerve activity and peripheral and central hyperexcitability, phenotypic changes in pain conducting pathways, secondary neurodegeneration, and morphological reorganization.^{12 127}

It is also recognized that episodic inflammation, and chronic inflammatory conditions, cause nerve injury, encouraging a broader appreciation of the heterogeneity of chronic pain aetiology.^{30 103} Here there are emerging data that implicate host defence mechanisms and powerful neuroimmune modulation involving peripheral and central immune cell activation in the initiation and maintenance of neuropathic pain.^{94 103 109 123}

Mechanistic and molecular-based understanding of chronic neuropathic pain has relied heavily on animal studies using few and approximated disease or mechanism-related models that emphasize key symptomatic characteristics (e.g. mechanical allodynia). Often these models involve peripheral nerve lesions, for example, sciatic nerve ligation, partial sciatic nerve transection, transaction of sciatic branches (spared nerve model), or ligation of L4 or L5 spinal nerves.¹² There are relatively fewer studies from other

disease-related models, for example, diabetic neuropathy or cytotoxic injury, to provide a balanced view about the ubiquity of key mechanisms. Nevertheless these studies, supported by emerging human genetic and empirical clinical observations, have highlighted a number of emerging therapeutic opportunities that reduce hyperexcitability in pain pathways, through block of abnormally active ion channels (voltage and ligand gated), direct inhibition of excitability (via GPCRs), or restoring normal neural phenotype and metabolism (e.g. neurotrophins).

Reduction of abnormal excitability

Ion channel (voltage and ligand gated) blockers

Hyperexcitability in small and large peripheral sensory nerves acts as an important driving mechanism for neuropathic pain and can account for the initiation and maintenance of central hyperexcitability.¹²⁷ Thus, block of local excitability using the local anaesthetic, lidocaine, reverses primary and secondary allodynia in neuropathic pain⁴¹ at concentrations that do not affect normal nerve conduction. This is due to the well-known activity-dependent increase in voltage-gated ion (sodium and calcium) channel affinity for local anaesthetics. Though peripheral excitability may be regulated by many mechanisms, selective depression of small and large fibre excitability appears to be a valuable path towards new therapies.⁷²

Changes in the expression and activity of several voltage-gated sodium, potassium, and calcium channels have been highlighted after nerve injury. Voltage-gated sodium channels are characterized by their primary structure and sensitivity to tetrodotoxin (TTX). A variety of TTX-sensitive (Nav1.3 and Nav1.7) and TTX-insensitive (Nav 1.8 and Nav1.9) channels are involved in regulating sensory neural excitability.^{32 71} Changes in the expression, trafficking, and redistribution of Navs, after inflammation or nerve injury, are considered to account for unstable oscillations of membrane potential, abnormal firing, and the generation of ectopic activity in afferent nerves.^{3 27} Mutations of Nav1.7 have been identified as the cause of burning pain in erythromelalgia,¹²⁵ whereas loss, or function mutations in these channels, leads to complete ablation of pain perception in humans, without any other notable pathology. Similar ablation of pain sensation has been observed in mice with Nav1.7-deficient sensory neurones.⁷⁹

Nav1.8 is uniquely expressed in small sensory neurones and may be down-regulated in small injured axons but up-regulated in adjacent uninjured C fibres and in human peripheral neuropathies.^{20 39} Nav1.8 appears important for the generation of spontaneous activity in damaged sensory axons,⁹⁵ and knockdown of Nav1.8 in models of neuropathic pain produces a marked reduction in abnormal pain responsiveness.⁴⁰ Recently, an Nav1.8 selective compound

(A-803467) has been described⁵¹ with a profile of activity in a variety of chronic pain models but surprisingly was ineffective in models of acute pain, suggesting that this channel makes little contribution to nociception in the physiological range.

Nav1.3 is also overexpressed in large A β fibres from chronic pain patients and in the spinal cord of pain models.^{20 43} This channel may also be an important substrate for oscillations in peripheral and central neurone excitability, which is considered to be important for neuropathic pain.

Among the major safety challenges in exploiting Na channel biology for neuropathic pain treatment is channel subtype selectivity comparing heart (e.g. Nav1.5) and CNS channels (e.g. Nav1.2). Some of these pitfalls are avoided by localized treatment of hyperexcitability with local anaesthetic formulations such as lidoderm. Emerging oral treatments include new sodium channel blockers such as lacosamide (also called Vimpat or harkoseride, Schwarz Pharma) and ralfinamide (NW-1029, Alipamide, Newron Pharma) with activity at both TTX-sensitive and TTX-resistant channels. These drugs have been shown to be anti-hyperalgesic in models of inflammatory and neuropathic pain.^{8 45 118} In addition, lacosamide is claimed to modulate collapsin response mediator protein 2 which may influence axon growth and neuronal plasticity.

Clinical benefits of lacosamide have been reported to include significant reductions in pain measures compared with placebo, achieved in some (but not all) phase 3 trials and accompanied by improvements in other efficacy measures including somnolence and quality of life (QOL) measures. Although reportedly well tolerated in the phase 3 trials, lacosamide was associated with high rates of adverse events and dropouts in trials in diabetic neuropathic pain.

Ralfinamide is another important therapeutic development blocking Na-ion channels and inhibiting the enzyme monoamine oxidase. This compound has shown a robust activity in a number of preclinical pain models. Clinical reports also suggest good tolerability overall with positive Ph2 outcome in a mixed population of neuropathic pain patients.¹³

Voltage-gated calcium channels are subdivided into two major categories: low-voltage-activated calcium channels (T-type channels) and high voltage activated. High-voltage-activated channels are further subdivided, based on pharmacology and biophysical characteristics, into L-, N-, R-, P-, and Q-types. Several have been shown to be prominently involved in pain regulation.¹²⁸ With respect to calcium channels, N-type channels are unique to neurones and critical for pain neurotransmission. Thus, deletion of the N-channel gene reduced inflammatory and neuropathic pain⁹⁶ whereas intraspinal block with the snail conopeptide Ziconotide (Prialt, Elan/Eisai),⁷⁶ or channel block with the orally available small molecule NMED-160 (Merck/Neuromed),¹⁰⁵ has shown efficacy across a range of chronic pain conditions

indicating the validity of this approach. Unfortunately, development challenges have halted the Ph2 delivery of NMED-160 whereas the technical limitations of intraspinal delivery of Prialt restricts the wide-spread usage of this approved agent.

Another highly validated approach targets the $\alpha 2$ delta-1 calcium channel subunit, the substrate for the anticonvulsant gabapentin and pregabalin, commonly used for neuropathic pain therapy. This subunit is important for channel assembly and its overexpression in small dorsal root ganglia (DRGs) and spinal neurones has been associated with allodynia in a number of specific pain models.^{56 67} The exact mechanism of action of gabapentin is unknown, although block of N-channel regulated-release of CNS neurotransmitters has been considered to be critical. More recent evidence suggests that gabapentin is transported into cells via an L-neutral amino acid transporter and acts to displace an endogenous ligand that regulates the trafficking of $\alpha 2$ delta-1 subunit to the neural membrane.⁴⁷ On the clinical side, emerging therapeutic differentiation in terms of bioavailability is claimed for the gabapentin prodrug XP 13512 (Xenoport/GSK) which is currently in Ph2 development.

Low-voltage-activated T-channels also appear important for pain transmission and as targets for pain therapy. Thus, they are expressed in superficial laminae of the spinal cord and in dorsal root ganglion neurones¹²⁸ and play a prominent role in regulating spinal excitability after repetitive C-fibre stimulation.⁴⁹ Moreover, nerve injury-induced hyperresponsiveness was reported to be blocked by the T-channel blocker ethosuximide⁷⁰ which also attenuated mechanical allodynia in animal models of vincristine and paclitaxel-induced neuropathic pain.³⁵

In addition to the voltage-gated ion channels described above, a number of ligand-gated ion channels are also being proposed as targets for neuropathic pain (NP) therapy. For example, the mammalian transient receptor potential (TRP) channels represents a large receptor family, subdivided into six subfamilies (for review see Richardson and colleagues),⁹² but attention has been focused mainly on TRPV1, TRPV3, and TRPM8. Many TRP channels are localized to sensory neurones and nearby keratinocytes, acting as temperature and mechanical transduction proteins. TRPV1 has been investigated in detail. This is a non-selective cation channel, gated by capsaicin, noxious heat ($>45^{\circ}\text{C}$), acidic pH (<5.3), and is regulated by a variety of inflammatory mediators (e.g. bradykinin and PGE2) and neuroregulators [anandamide and nerve growth factor (NGF)]. It is emerging as an important molecular focal point that is thought to play an important role in neural sensitization caused by mediators of inflammation and nerve injury.^{91 117}

Current analgesia strategies are aimed at developing either TRPV1 agonist or antagonist drugs to attenuate excitability in sensory fibres. TRPV1 agonists induce receptor desensitization or a reversible sensory nerve terminal

degeneration due to prolonged cation influx into the nerve, osmotic damage, and metabolic collapse.¹¹¹ In this regard, topical formulation of high-dose capsaicin (e.g. Transacin from NeurogesX) has been shown to be efficacious in a number of neuropathic pain conditions including Ph3 studies in PHN patients.⁹

On the other hand, antagonists aim to selectively inhibit peripheral nerve fibre activity by block of TRPV1 signal transduction. Supporting these approaches, competitive (AM G9810 and AMG628)^{38 121} and non-competitive TRPV1 antagonists (DD161515)³⁷ block chemical and thermal pain sensitivity, supporting the emergence of a novel therapy. Indeed, recent clinical studies in volunteers have shown that oral SB705498 (GSK) attenuated capsaicin and UVC-induced pain and hyperalgesia,¹⁶ without notable side-effects. These studies herald the way for a number of Ph2 trials for competing antagonists (e.g. GRC 6211 Lilly/Glenmark; NDG6243 Merck/Neurogen; AZD1386, AstraZeneca). However, others have noted the occurrence of hyperthermia with this class of antagonists both in animal and in human volunteer studies.³⁸

Other TRP channels (TRPV3, TRPV4, TRPA1, and TRPM8) that also act as temperature transducers have been suggested to be involved in pain particularly when sensitized by a pathophysiological environment. TRPA1 is co-localized with TRPV1, is activated by capsaicin, and like TRPM8, can also be sensitized by inflammatory mediators and nerve injury to produce cold-induced burning pain.^{7 83 87} A TRPA1 selective antagonist HC030031 has been claimed to reduce pain signalling in animal models.⁷³ TRPV3 is also found in keratinocytes and sensory neurones²⁸ and appears to be up-regulated in diabetic neuropathy. So far there are few available chemical tools to help characterize the functions of these receptors, although TRPV3 ligands (Pfizer/Hydra) appear to be in early development.

Neuronal nicotinic receptors (NNRs) are part of a complex family of ligand-gated ion channels. These comprise five subunit proteins that form a cation permeable ion channel. Models of chronic injury suggest that there is significant overexpression of key subunits including the $\alpha 7$ NNR and $\alpha 4$ and $\alpha 5$ subunits. This has supported the therapeutic potential of NNRs in pain control.^{24 119} Thus, nicotinic receptor agonists (epibatidine, ABT-594, Abbott) selective for these subunits, particularly compared with $\alpha 4$ $\beta 2$ have demonstrated antinociception in several models of pain including neuropathic pain.⁷⁵ Several sites of action have been proposed including supraspinal sites via descending monoaminergic and muscarinic pathways, and actions on peripheral sensory neurones. Earlier clinical evaluation of ABT-594 revealed inadequate tolerability, but more recent compounds such as TC 6499 (GSK/Targacept) and ABT-894 (Abbott Pharma) are progressing in clinical phases 1 and 2, respectively, towards a diabetic neuropathy pain indication.

The excitatory transmitter glutamate plays an important role in the initiation and maintenance of neuropathic pain.

Glutamate acts through a variety of receptors which include the *N*-methyl-D-aspartate receptors (NMDA), the alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA)/kinate receptors, ionotropic glutamate receptors (iGluRs), and GPCR coupled, metabotropic glutamate receptors (mGluRs) that are coupled with ligand-gated ion channels. Injections of glutamate or metabolically stable receptor-selective agonists, such as NMDA, AMPA, and kainate, cause a pro-nociceptive response, whereas the administration of iGluR and mGluR antagonists attenuates pain.^{80–112} Encouragingly, tezampanel (NGX426; Torrey-Pines), an AMPA kinate antagonist formulated for i.v. administration because of poor bioavailability, has shown positive outcome in several clinical trials^{99–100} but with dose-limiting side-effects including blurred vision and sedation. The development of an oral prodrug is in progress with the promise of improved bioavailability.

NMDA antagonists also show robust attenuation of pain in animal models but induce a number of side-effects (sedation, confusion, and motor incoordination) and thus appear to have insufficient therapeutic margin.⁸⁴ In an attempt to avoid these side-effects, specific blockers of NMDA receptor subtypes (NR1 and NR2) are being developed directed at the strychnine-insensitive glycine_B modulatory site. This site modulates the NMDA channel during sustained receptor stimulation, which is considered to occur during chronic pain. Selective NR1-glycine (NR1-Gly) site antagonists have been claimed to reduce pain with reduced side-effects.^{23–88} However, clinical experience has not confirmed this. GV196771 (GSK) did not show efficacy against clinical pain, possibly due to inadequate penetration into the CNS.¹²⁰

An alternative approach has targeted another NMDA receptor subtype, the NR2B receptor,⁴⁰ which has a specific distribution in sensory pathways. Block of this receptor has also been claimed to produce antinociception (ifenprodil, traxoprodil, CP-101606) with reduced side-effects.¹¹³ To date, traxoprodil has advanced into phase 1 safety and efficacy studies for acute ischaemic stroke, and there is little information about possible development in pain. On the other hand, other NR2B programmes including RGH 896 (Gideon Richter) and EV101 (Evotec) are continuing in phase 2 and are evaluated for cognition and neuropathic pain.⁴⁰

Metabotropic glutamate receptors, particularly mGluRs 1 and 5, have been reported to play a key role in sustaining heightened central excitability in chronic pain with minimal involvement in acute nociception. Thus, spinal administration of selective agonists such as dihydroxyphenyl-glycine produced allodynia, whereas mGluR5 has been shown to be significantly over-expressed in some chronic pain models.⁴⁸ Peripheral mGluR5 receptors have also been claimed to modulate pain. In keeping with this, several mGluR5 antagonists (MPEP and SIB 1747) have shown robust efficacy in neuropathic pain models.^{29–130} In addition, several compounds (ADX10059 and ADX4861)

have provided validation of this target in several clinical pain conditions including migraine and fibromyalgia⁵⁰ whereas additional studies, for example, AZD2066 (AstraZeneca), are currently in progress, Ph1 and 2.

Metabotropic Group II receptors (mGluR2 and 3) also modulate pain transmission. The mGluR2 is located in sensory neurones and presynaptic nerve terminals, whereas mGluR3 is found all over the brain. MgGluR3 can be selectively increased in spinal dorsal horn neurones after peripheral injury.¹⁰ MgGluR2 and 3 receptor activation appears necessary to reduce nerve terminal excitability and to modulate pain transmission since treatment with the agonist L-acetyl carnitine reduced inflammatory hyperalgesia and mechanical allodynia and increased the expression of mGluR2 and 3. The effects of L-acetyl carnitine were attenuated by LY379268, an mGluR2 and 3 antagonist.¹⁵

Enhancing inhibition

Cannabinoids and monoamine pathways

Other emerging approaches to attenuate hyperexcitability in pain circuitry are to enhance cellular inhibitory mechanisms. These include targeting cannabinoid receptors or enhancing central monoaminergic inhibitory control.

There are two major cannabinoid receptors, CB1 and CB2, associated with pain modulation. CB1 receptors are widely distributed in the CNS and peripheral sensory neurones, whereas CB2 receptors have been found in peripheral tissues including tissues of the immune system and keratinocytes with limited expression in sensory and CNS cells.³⁷ Several fatty acids (e.g. anandamide, 2-arachidonylglycerol, and palmitoylethanolamide) have been identified as endogenous ligands for these receptors whereas specific antagonists such as SR141716A, SR147778 for CB1 and SR144428 for CB2 have been used to characterize receptor function and support the validation of CBs in models of chronic pain.³⁶

On the basis of current evidence, the efficacy of cannabinoids towards pain modulation is mediated mainly through CB1 receptors located in both the peripheral and CNS. Several clinical studies have shown that cannabinoids, such as delta(9)-tetrahydrocannabinol (THC) or Sativex (THC plus cannabidiol: GW Pharma), reduce neuropathic pain,⁸¹ but these compounds also produce adverse effects such as euphoria, dizziness, and sedation at therapeutic concentrations.¹¹ However, selective targeting of the peripheral cannabinoid CB1 receptors appears to reduce CNS side-effects while maintaining significant pain relief. Indeed, studies in which the central CB1 receptor was deleted suggest significant analgesic efficacy can be retained through peripheral CB1 activation.² Furthermore, localized or topical administration of non-selective agonists such as HU210 or KDS2000 (Kadmus Pharma) or the oral administration of a CB1 agonist with limited CNS

availability such as CT3 (ajulemic acid, CT3: indevus Pharma), produced analgesia both in pain models^{31 92} and in a clinical test at a dose which caused minimal CNS side-effects.⁵⁴ Another oral, peripherally selective cannabinoid agonist (AZD 1940 AstraZeneca) is currently undergoing clinical validation.

Interestingly, CB2 selective agonists (e.g. HU-308, HU-210, CP55940, AM1241, and GW405833) have also been shown to modulate chronic pain.^{60 68 116} It is unclear how the analgesic effects of CB2 agonists are produced since few CB2 receptors are found in the CNS or on sensory neurones.¹⁰⁶ However, *de novo* expression of CB2 receptors occurs in central glial cells after peripheral nerve lesions.¹²⁹ It is therapeutically significant that CB2 agonists have not shown CB1-like side-effects.

An alternative approach to utilize the endogenous cannabinoid systems is via inhibition of fatty acid amide hydrolysis (FAAH), the major degradation pathway for endogenous cannabinoids.²² Data supporting this show that mice lacking this enzyme,²¹ or treatment with FAAH inhibitors, such as URB597 and OL135, significantly elevated brain anandamide and increased pain threshold in pain models.⁶⁴ Moreover analgesic synergy has been reported to occur between opioid and cannabinoid systems¹⁷ suggesting that in a clinical pain setting, greater therapeutic benefit may be achieved by combinations of these mechanisms.

The successful treatment of neuropathic pain with amitriptyline and duloxetine has stimulated further interest in exploring mechanisms and drugs (e.g. venlafaxine and bupropion) that enhance monoamine pathways in the CNS. Since the effects of these SNRI drugs in chronic neuropathic pain appear to be independent of antidepressant activity, an attractive hypothesis is that they reinforce descending inhibitory pain circuitry. Other types of monoamine inhibitors, for example, SNRI (fluoxetine and paroxetine), selective serotonin reuptake inhibitor (SSRI) (mirtazapine), or norepinephrine reuptake inhibitor (NRI) (reboxetine), require further evaluation.⁷⁴ However, it has been concluded that antidepressants represent useful tools in neuropathic pain treatment and must still be considered as first line treatments. Without head-to-head comparisons between antidepressants and other analgesics, it is not possible to provide real evidence-based treatment algorithms for neuropathic pain.¹⁰⁷

As mentioned earlier, there is emerging evidence for neuroimmune modulation in the aetiology of neuropathic pain. In particular, neuroglial cells (microglia, astrocytes, DRG satellite cells, and Schwann cells) have been highlighted as key cellular elements in these processes. Importantly, both trafficking of peripheral leucocytes into the CNS and activation of resident glial cells contribute in the aetiology of neuropathic pain. Glial cell activity is important in pain amplification as many of these cell types regulate neuronal activity but also make close-junctional connections with each other, providing a means of spreading excitability changes beyond the boundaries of spinal

segmental input. Also of importance is that glia secrete a number of mediators (nitric oxide, neurotrophins, IL1 β , TNF- α , and free radicals), some of these are associated with synaptogenesis but also with the plasticity that causes changes in spinal and afferent neuronal excitability including secondary neurodegeneration and the loss of inhibitory inter-neurons.⁷⁸

The release of inflammatory mediators is regulated via glial receptors for ATP, kinins, prostanooids, NMDA, and a variety of chemokine receptors, which may also modulate spinal excitability.^{124 126} A number of compounds claimed to stabilize and reduce glial activity have been shown to attenuate evoked pain in models of neuropathic pain. These include propentofylline,^{89 110} minocycline,¹²⁴ and acetyl L-carnitine. Interestingly, ibuprofen (Avigen 411), a drug used clinically for respiratory disorders, has recently been shown to be efficacious in neuropathic pain⁶² through reduction of neuroglial activation. Although ibuprofen is known as a PDE4 inhibitor, this does not readily explain its actions at neuroglia and for the most part the actions of the aforementioned drugs that attenuate glial activity are not well understood.

Glial regulation may also be achieved through receptor modulation. In this respect, a number of purinergic²⁶ receptors have been highlighted. For example, P2X7 and P2X4 receptors are expressed in microglia and satellite cells and are up-regulated after peripheral nerve injuries.^{14 115} Deletion of the P2X7 receptor-gene produced a complete absence of mechanical and thermal pain in mice¹⁴ whereas the selective P2X4 antagonist TNP-ATP attenuated mechanical allodynia.¹¹⁵

Other major inflammatory products such as chemokines and their receptors have important roles in neuropathic pain. The major chemokines and their respective receptors are MCP1/CCR2, MDC/CCR4, RANTES/CCR5, fractalkine/CX3CR1, and SDF-1 α /CXCR4. Specific chemokines, for example, TNF- α , IL-8, and patterns of chemokine release^{58 65} have been strongly associated with a number of neuropathic pain conditions.^{63 102} With respect to chemokine receptors, CCR2, CXCR4, CCR4, and CX3CR1 have been shown to be expressed in glial and sensory neurones, and preliminary data have shown that block of CX3CR1 by a receptor-neutralizing antibody induces powerful anti-allodynic effects. In addition, CCR2 has emerged as an important target being powerfully expressed in spinal glia and DRGs after peripheral nerve injuries and activated by its ligand MCP-1 to activate microglia and increase sensory fibre excitability.¹ In support of this, deletion of the CCR2 gene blocks glial cell recruitment and attenuates neuropathic pain.¹ Although these preclinical data seem compelling, it should be noted that CCR2 antagonists have been in development (INCB3284, Pfizer/Incyte and MK-0812 Merck) for treatment of rheumatoid arthritis but have not proved to be clinically efficacious.

Although many cytokines appear to have a detrimental role in initiation and maintenance of neuropathic pain,

some cytokines appear to be beneficial. Thus, a gene therapy approach, using viral and non-viral vectors (AV-333), has been used to increase spinal IL10 production. This experimental treatment reversed the mechanical allodynia evoked in a model of neuropathic pain.⁷⁷ These are instructive observations as they suggest that care must be given to maintaining a positive therapeutic balance when modulating the neuroimmune products that regulate chronic pain.

Restoring the neural phenotype

As mentioned earlier, a variety of phenotypic changes have been identified in cellular elements of the pain pathways contributing to neuropathic pain and associated dysesthesias. These changes drive alterations in expression of receptor and ion channel proteins, alterations in cellular neurochemistry and patterns of released transmitters, and synaptic reorganization that impacts on normal sensory function. These changes can be regarded as critical elements of the disease processes that maintain the chronic pain state. Phenotypic changes are understood to occur through altered dynamic interactions between intracellular and extracellular regulators, the latter often being contributed by supporting cells such as keratinocytes, Schwann cells, and neuroglia or by migrating cells such as macrophages and leucocytes that release neurotrophins. Modulation of neurotrophin regulation is seen as an attractive therapeutic possibility to address both chronic pain symptoms and disease progression and is being pursued by developing both small molecule and biological approaches.

Neurotrophins and their receptors

Neurotrophins represent an important family of regulatory proteins essential for sensory nerve development, survival, and the determination of neurochemical phenotype critical for the regulation of excitability.^{42 131} Several neurotrophins have been identified including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin 3 (NT3), NT 4/5. Each neurotrophin binds with high affinity to a receptor tyrosine kinase (trk): NGF to trkA, BDNF and NT4/5 to trkB, and NT3 to trkC. NT3 also binds with trkA and trkB. Mature neurotrophins also bind to a structurally distinct receptor p75 which affects neuronal development through downstream signalling. Neurotrophins arise from proneurotrophin precursors after extracellular cleavage by metalloproteinases and plasmin. It is notable that proneurotrophins may signal through the p75 receptor in a manner that opposes the effects of neurotrophins, for example, to produce apoptosis rather than cell survival.⁶⁶

NGF is one of the most studied neurotrophins. It is a key regulator of sensory neurone excitability and an important mediator of injury-induced nociceptive and neuropathic pain.^{46 93 114} NGF acts via trkA and p75 to

activate a number of other kinase-pathways, for example, p38 kinase leading to altered gene transcription and the increased synthesis of sensory neuropeptides (substance P and CGRP), ion channels (TRPV1, Nav1.8, and ASIC3),^{34 52 69} membrane receptors such as bradykinin and P2X3,⁹⁰ and structural molecules including neurofilament and channel anchoring proteins such as the annexin light chain p11.

Increased expression and release of NGF have been demonstrated, for example, in UV and surgical injury⁴² and human disease conditions including arthritis, cystitis, prostatitis, and headache.^{5 44 101} Localized administration of exogenous NGF induces thermal and mechanical hyperalgesia in animals and in humans^{4 108} which is considered to be due in part to mast cell degranulation and by directly increasing sensory neuronal excitability.

Few NGF antagonists have been reported, but ALE0540 and PD90780 which inhibit NGF binding to trkA and p75, respectively, have shown efficacy in chronic pain models.^{18 86} In addition, the therapeutic utility of targeting NGF has also received clinical confirmation since humanized anti-NGF monoclonal antibodies (mAb) RN624 (Pfizer/Rinat) and AMG403 (Amgen) have been reported to be efficacious in reducing pain and improved mobility in osteoarthritis.⁶¹ Anti-NGF mAb therapy thus appears as an attractive therapeutic approach with further validation being pursued in other indications including PHN and low back pain.

BDNF is another important neurotrophin released from neuroglial and sensory cells and whose expression can be regulated by NGF after painful nerve injury.⁹⁷ In the spinal dorsal horn, BDNF increases spinal excitability by direct neural excitation, activation of a signalling cascade via the phosphorylation of NMDA receptors, and via altered regulation of the neural chloride-ion transporter that contributes to pain hypersensitivity.¹⁹ In addition, spinally administered BDNF causes thermal and mechanical allodynia whereas anti-BDNF neutralization or trkB IgG administration reduces nerve injury hypersensitivity in animal models.^{25 55}

Finally, GDNF represents an extensive family of ligands and membrane receptor complexes that have an important role in regulating peripheral and central neural phenotypes. GDNF-related ligands include neurturin and artemin, which act via the complex RET tyrosine kinase receptor and co-receptors GFRalpha1, alpha2, alpha3, and alpha4. GDNF has been shown to have neuroprotective and restorative properties in a number of neurodegenerative and neuropathic pain states.⁹⁷ Specifically, GDNF and more recently artemin treatment have been shown to restore peripheral sensory neurone function,¹²² including peptide and ion channel expression patterns, after peripheral nerve injury accompanied by an attenuation of pain behaviours.⁹⁷ Unfortunately, clinical observations using GDNF have shown unacceptable side-effects such as weight loss and allodynia which has discouraged therapeutic development.⁸²

Finally, it is worth mentioning another biological approach to neuropathic pain that involves gene therapy. Thus, SB-509 (Sangamo Bioscience), a plasmid that expresses an engineered gene transcription factor (a novel DNA-binding zinc finger protein), binds the endogenous VEGF-A promoter gene to improve microvascular regrowth and thereby promote peripheral nerve regeneration and attenuate diabetic neuropathic pain. This concept has been supported by preclinical studies^{57, 104} and is currently in phase 2 for clinical validation.

Summary

Neuropathic pain therapy remains an enormously challenging area of unmet medical need, despite the increases in knowledge of its aetiology and cellular mechanisms. Many details are still lacking, particularly with respect to the time-related changes underlying pain progression and the complexity of overlapping mechanisms and comorbidities. Mechanism-based approaches have highlighted key areas for intervention including the reduction of peripheral and central hyperexcitability through a number of molecular targets. Among these targets are a variety of ion channels, whose expression changes during neuropathic pain and where block has shown therapeutic utility. These include sodium (Nav1.7, Nav1.8, and Nav1.3) and calcium channels (Cav2.2 and α 2-delta subunits) and a number of ligand-gated channels of importance in the processes of neural sensitization (TRPV1, TRPM8, and NNRs). Other approaches which include the modulation of peripheral excitability via activation of CB1 receptors, reduction of spinal excitability through block of excitatory glutamate receptors (mGluR5 and AMPA), block of activated spinal neuroglia (CCR2 and P2X7), or increasing spinal inhibition by enhancing monoaminergic activity, all offer exciting opportunities currently being validated in the clinic. Finally, the emergence of biological approaches, for example, antibodies, siRNA, and gene therapy, offer powerful therapeutic possibilities to treat neuropathic pain and other associated neurological dysfunction.

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