Neuropathic Pain: Some Clues for Future Drug Treatments

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Abstract: Neuropathic pain is still far from being adequately dealt with. Under this name, several clinical entities have been considered and most of them only share several painful ailments. At present, the available treatments can only alleviate the pain of roughly half of the patients, and their effectiveness is often limited by the appearance of the intolerable side effects. In this review, we will consider the pathophysiology of neuropathic pain to understand the basis of pharmacological treatments that are currently being investigated. Some examples of these drugs will also be considered.

Keywords: Pain, neuropathic pain, analgesics, pathophysiology, allodynia, hyperalgesia, experimental models, new drugs.

SETTING THE PROBLEM

If we ask someone to define pain, it could not only be difficult, but also incomplete, because each person feels it in a different way. The International Association for the Study of Pain (IASP) has defined it as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" [1]. This is a rather bizarre definition that adds little to our understanding of what pain is. However, the statement clearly establishes some the most important features of pain, such as its subjective character, the existence of an emotional component added to the sensory stimulation and the presence of pain even if the organic injury is apparently absent. This is especially important, as a lot of unnecessary suffering might be avoided if the need of an anatomical injury is redundant to believe that a patient is in pain. Additionally, emotional aspects of pain should always be considered, especially in those patients afflicted by chronic pain. Unfortunately, most of these patients may only expect partial relief with current therapies, and optimal treatment will never be fully reached if psychological aspects are ignored [2].

In the clinical setting, pain has been classified as acute and chronic, but this is rather a disappointing way of considering such a complex issue. Temporal criteria may not be the best method to make therapeutic choices or preparing management plans. In this way, it seems better to follow the suggestion of Scholz and Woolf [3], who classify pain in three categories: nociceptive, inflammatory and neuropathic pain (NP). The first is triggered by noxious stimuli acting on a specialized high-threshold sensory apparatus and usually stops after the stimuli disappear. It is an important mechanism of survival that tries to preserve the body from any harmful stimuli that may cause tissue damage. Therefore, it is also known as physiological pain and is, in some way, the unique pain considered in most physiology textbooks. Nociceptive pain has no long-lasting consequences and it has no other interest except knowing how our body maintains its integrity. It is, in fact, an old evolutionary mechanism to preserve our life.

A rather different picture appears when we consider inflammatory pain. Here the injury is severe enough to elicit the release of inflammatory mediators –cytokines, growth factors, kinins, purines, amines, prostanoids, ions, including protons- from damaged tissue and inflammatory cells [4,5]. These mediators activate, directly or indirectly, the nociceptors, and this activation evokes pain and/or produces the sensitization of the peripheral sensory nervous system which will turn into a source of painful stimuli for the patient. An example is the postoperative pain that follows a surgical procedure: the mechanisms that are set on physiologically to repair the damage induced by the surgeon produce unavoidable pain. In most of the cases, inflammatory pain self-limits and disappears once the injury is healed, but it persists over time in some patients. Pain arising from chronic inflammatory conditions, such as rheumatoid arthritis, should also be included in this category. Therefore, from the pathophysiological point of view, inflammatory pain is not synonymous of chronic pain, as it includes both acute and chronic conditions.

When the nervous system is injured at peripheral or central sites, the resulting pain is labeled as neuropathic. It is rather different from nociceptive and inflammatory pain and, hence, its management should be established on different grounds. First, NP is difficult to treat as it is generally resistant to drugs, such as opioid and non-steroidal anti-inflammatory drugs (NSAIDs), that may provide relief in patients afflicted with nociceptive or inflammatory pain. As a consequence, the development of new analgesics that may relieve these patients is urgently needed. Nevertheless, the achievement of this goal should follow rather different approaches than those followed in the 'traditional' analgesic research.

THE IDENTITY OF NEUROPATHIC PAIN

Sometimes, confusion arises when considering two closely related entities: neurogenic and NP. The International Association for the Study of Pain [1] has defined neurogenic

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pain as "Pain initiated or caused by primary lesions of dysfunction or transitory perturbation in the peripheral or central nervous system." An almost identical definition is applied to NP, the only difference being the lack of mention of "transitory perturbation". Furthermore, for some authors [6] the definition is too broad, and under dysfunction many nociceptive and psychogenic conditions might be included. They suggest, for the sake of simplicity, an amended definition of NP: "Pain due to a primary lesion of the peripheral or the central nervous system" [6]. Then, the term "neurogenic pain" is confined to classical neurological painful condition where neuropathy may be difficult to establish, such as glosopharyngeal or trigeminal neuralgia. The reversibility of symptoms should not be considered as a criterion, as long-standing conditions, such as postherpetic neuralgia (PHN), may subside over time [7]. Besides these accurate differences, the truth is that the medical literature rarely distinguishes between neurogenic and NP syndromes. Although we will use the latter in our review, readers should be informed that both terms are generally considered as interchangeable in the medical literature.

From the clinical point of view, NP is characterized by the existence of spontaneous (i.e. not stimulus-evoked) pain and abnormal stimulus-evoked pain. When a stimulus that usually causes mild pain is perceived by patient as producing severe pain, this situation is called hyperalgesia. Depending of the nature of the stimulus, the resultant condition is known as heat, cold or mechanical hyperalgesia. However, in some cases painless stimuli (such as the rubbing of clothing) are felt as painful, and this situation is known as mechanical allodynia. Nonetheless, such symptoms may also appear in inflammatory pain (e.g. sunburn). Mechanical allodynia may be very distressing for some patients and many of them may cope with their clinical condition only when this situation is adequately dealt with. Besides hyperalgesia and allodynia, there are other evoked sensory phenomena, such as paresthesia (abnormal sensation, whether spontaneous or evoked, not unpleasant) or dysesthesia (abnormal sensation, whether spontaneous or evoked, unpleasant) [8].

The conditions that may cause NP are summarized in Table 1. Treatment is often unsuccessful (see below), makes the life of the patients miserable, and some of them even commit suicide. There is an urgent need for finding new and effective therapies for NP, and research on the mechanisms that are responsible for such symptoms may provide the desirable data to establish the best pharmacological targets. Accordingly, the research of new drugs should be directed to prove their efficacy in the best experimental models that are available.

ON THE EXPERIMENTAL MODELS OF NEUROPATHIC PAIN

When new drugs with potential usefulness in NP are evaluated, both the experimental models and the associated behavioral responses should be considered. For medicinal chemists, a basic knowledge of them is desirable in order to consider critically the results of pharmacological testing. In the next paragraphs, a summary of the experimental models often used is offered.

Table 1. Classification of Neuropathic Pain by Primarily Peripheral or Central Nervous System Injury

Peripheral nervous system
• Traumatic injury of nerves, root nerves or nerve plexus: direct (trauma, avulsion) or indirect (compression, entrapment).
• Ischemic neuropathy.
• Polyneuropathy: hereditary, metabolic (diabetes), toxic (drugs, environmental substances, toxins), inflammatory, infectious, nutritional (poisoning or vitamin deficiency).
• Stump and phantom pain (after amputation).
• Herpes zoster and postherpetic neuralgia.
• Cancer-associated neuropathy: nerve invasion of the tumor, iatrogenic (after surgery, radiation therapy or chemotherapy), paraneoplastic syndromes.
Central nervous system
• Traumatic: spinal cord injury
• Vascular: stroke by infarct of hemorrhage.
• Degenerative: multiple sclerosis, syringomyelia/syringobulbia.
• Epilepsy
• Space-occupying lesions.

Table 2. Peripheral Mechanisms of Neuropathic Pain (After Nerve Ligature)

- Ectopic and spontaneous discharges: great increase in the level of spontaneous firing in the injured afferent neurons
- Ephaptic conduction: direct coupling between axons.
- Alterations of the ion channel expression (sodium and calcium channels): it increases the excitability of the neurons and influences the generation of hyperalgesia and allodynia.
- Collateral sprouting of primary afferent neurons: due to NGF release of skin sources.
- Sprouting of sympathetic neurons (noradrenergic perivascular sympathetic postganglionic axons) into the DRG: this sympathetic input could activate the neurons because the terminals of the sprouted neurons form functional synapse-like structures with the cell bodies.
- Nociceptor sensitization: it is caused by the release of inflammatory mediators with a concurrent decrease in the threshold for stimuli and an increased response to suprathreshold stimuli.

Several models imply a surgical intervention on the sciatic or the spinal nerves. First, the nerve may be fully cut or ligated, whose functional consequences should be similar to an amputation. Second, nerve may also be injured by a partial nerve lesion (PNL), made by a tight ligature around a part of the nerve fascicles [9] or by a chronic constriction injury (CCI) produced by placing several loose ligatures around the sciatic nerve [10]. A third possibility is the well-known Chung model where a tight ligature of a spinal nerve (SNL) or transection of one or several dorsal roots is performed [11]. NP may also be a consequence of an ischemic lesion induced photochemically [12].

Several behavioral indices, which are a consequence of the involved mechanisms [13], are used to analyze NP. Most of them use stimulus-evoked pain [14]. Stimulus-independent pain (spontaneous pain) depends, mainly, on the spontaneous activities of the nociceptor C-fibers and large myelinated A-fibers [15]. However, it is difficult to

study spontaneous pain in injured animals and therefore is rarely used in pharmacological testing. By contrast, stimulus-evoked pain may be properly evaluated measuring thermal hyperalgesia and mechanical allodynia (sometimes cold-induced).

As explained above, hyperalgesia to noxious thermal stimuli is an increased response to a stimulus which is normally already painful, because of the hyperexcitation of the structures that subserve nociception. As a consequence, injured animals exhibit shorter latency responses (withdrawal from stimuli) than those that have been sham treated. Hyperalgesia may be mechanical (dynamic, static or punctate), thermal or chemical. Dynamic hyperalgesia is the consequence of an increased central response to Aβ-fiber input [14]. Conversely, allodynia to mechanical (application of von Frey hairs) or cold stimuli is a result of painless stimuli, and it due to the fact that elements of the sensory nervous system, which normally signal innocuous sensation, begin to encode painful stimuli. There are two types of mechanical allodynia: static allodynia, signaled by capsaicin sensitive Aδ-fibers; and dynamic allodynia, signaled by Aβ/capsaicin-insensitive Ad-primary sensory neurons [15].

There is another sign of pain, the excessive grooming, which is still under discussion, because skin irritations or itching can induce this behavior. The other way to study NP is the autotomy behavior (self-mutilation of the toes and foot of the injured limb) that has been used in the past but now is considered as a controversial model. For some authors, this behavior is a response to the total motor and sensory denervation of the hind-paw rather than the pain [16].

Most of the models proposed in the previous paragraphs have demonstrated to be reproducible and sensitive to several drugs used in NP. However, some authors are reluctant to accept that they are actually modeling the clinical picture in humans [6]. At the pharmacological level, some effective drugs in animal models, such as mexiletine [17] or dextrorphan [18], are of limited value in patients [6]. Nonetheless, others, like amitriptyline or gabapentin, are useful in both experimental and clinical NP. There is not a clear-cut answer to these discrepancies, and those working in the field should consider the clinical relevance of each animal pain model and be cautious in extrapolating the results of laboratory studies to the clinical setting.

PATHOPHYSIOLOGY OF NEUROPATHIC PAIN

After nerve injury, several physiological changes can be observed in the central and peripheral nervous systems (Table 3). The clinical counterparts of such changes are the appearance of positive (abnormal, spontaneous or evoked sensations) and negative (sensory deficits) sensations [19]. Accordingly, it is not infrequent to find hypoesthesia (diminished sensations) surrounded by hyperalgesic skin. In the periphery, ectopic (abnormal) and spontaneous discharges, abnormal nerve conduction, alterations of the ionic channel expression, collateral sprouting of primary afferent neurons, sprouting of sympathetic neurons and nociceptor sensitization mainly appear.

The *ectopic and spontaneous discharges* are expressed as a large increase in spontaneous firing in the afferent neurons linked to the injury site, which originates in the dorsal root ganglia (DRG) and along the nerves [20, 21]. Two populations of afferent fibers develop ectopic activity: the injured sensory neurons themselves and their uninjured neighbors [22]. These abnormal discharges can be spontaneous (due to instability of the membrane potential) or caused by undetectable stimuli.

Table 3. Mechanisms of Neuropathic Pain in the Central Nervous System (After Nerve Ligature)

- Central sensitization: appearance of "wind-up" or increasing response to repeated C-fiber volleys that may contribute to hyperalgesia. The key receptor in this process is the NMDA receptor.
- Spinal reorganization: the Aβ-fibers (large myelinated afferents that convey touch perception) sprout into lamina II of the dorsal horn, which is normally innervated by C-fibers), and (small afferent fibers), and establish functional synaptic contact with second-order neurons, then low-threshold non-noxious inputs from the Aβ-fibers can be interpreted as nociceptive in origin.
- Cortical reorganization: changes in the circuitry of some neurons in brain centers may follow the persistence of nociceptive inputs.
- Changes in inhibitory pathways: reduction of the inhibitory control over dorsal horn neurons through several mechanisms.
- Neuropeptide plasticity: consequence of phenotypic switch of primary afferents and characterized by an altered expression of neuropeptides and changes in their receptor levels in the dorsal horn.

The *alterations of the ion channel expression* implying the increase in the excitability of the neurons are the cause of ectopic discharge and may influence the appearance of hyperalgesia and allodynia. The involved ion channels are Na^+ and Ca^{2+} channels. There occurs *de novo* synthesis (up-regulation) of rapidly repriming III tetrodotoxin-sensitive (TTX-S) channels and down-regulation of tetrodotoxin-resistant (TTX-R) channels (SNS2/NaN and SNS/PN3) in the cell bodies and in the terminal neuroma of peripheral nerves following nerve injury [23,24]. Furthermore, there is a loss of high-voltage activated N-type channels, but not P- or Q-type [25]. These alterations have therapeutic interest and will be considered again in the last section of this review.

Collateral sprouting of primary afferent neurons means that these neurons spread in their vicinity and eventually establish new synapses. It is mainly due to NGF release of skin sources [26]. *Sprouting of sympathetic neurons* (noradrenergic perivascular sympathetic postganglionic axons) into the DRG forms baskets around the large diameter neurons; this sympathetic input could activate the neurons because the terminals of the sprouted neurons establish functional synapses-like structures with the cell bodies [27]. The induction of the sprouting is a consequence of NGF action at the level of the DRG, where the levels of mRNA are increased after nerve injury [28].

Nociceptor sensitization: it is caused by inflammatory mediators, like amines, prostaglandins, leukotrienes and bradykinins, that are released after injury [29]. When it occurs, there is a decrease in the threshold of stimuli and an increased response to suprathreshold stimuli.

Table 3 summarizes some of the central nervous system (CNS) mechanisms of NP. The main central mechanisms are

sensitization of spinal nociceptive neurons, neuropeptide plasticity, spinal reorganization, cortical reorganization, and changes in the inhibitory pathways. We will briefly refer to each of them.

Some experimental evidences, reviewed by Dickenson *et al.* [19], suggest that NP may arise, at least in part, from the central compensations for the loss of normal sensory inputs. Ongoing activity from peripheral nerves may evoke greater release in primary afferent transmitters and activation of N-methyl-D-aspartate (NMDA) receptors is required for the induction as well as the maintenance of NP [30]. It seems reasonable to accept that ectopic peripheral activity is amplified and enhanced by spinal mechanisms mediated by glutamate receptors. These mechanisms of compensation may be the underlying functional mechanisms of NP at CNS, but structural changes may also occur. For instance, Woolf *et al.,* [31] have shown that Aβ fibers which transmit touch sensation may sprout into the dorsal horn and make new connections with nociceptive fibers. Consequently innocuous mechanical stimuli are felt as painful ones, i.e. allodynia. These reorganizations of cell connectivity in the spinal cord would explain why pharmacological therapy is frequently ineffective against mechanical allodynia.

Sensitization of spinal nociceptive neurons after initial nerve damage by ongoing pathology, and/or ectopic activity in injured fibers is likely to be an important factor of NP [14]. This sensitization is characterized by the appearance of "wind-up", i.e. an increased response to repeated C-fiber volleys that may also contribute to hyperalgesia [32, 33]. “Wind-up” may be a consequence of an increased nociceptor drive or a loss of inhibition after nerve injury. Any of them leads to an exaggerated dorsal horn response to Aβ-fiber input and, consequently, to mechanical (tactile) hyperalgesia. Although this sensitization is related to the well-known long-term potentiation described in the brain and also in the spinal cord, studies in rats with damaged nerves have failed to find such changes in the spinal cord, probably because of an already preexisting elevated level of excitability [19]. This hyperactivity tries to compensate the decrease of ongoing peripheral activity with deleterious consequences. The key mechanism in this process is the activation of the NMDA receptor [18,34,35]. In fact, the activation of NMDA receptors is needed for the induction and maintenance of pain after nerve damage [30, 36]. It must be remembered that these receptors will only operate when the underlying level of excitability is high, as probably occurs when trying to compensate for the peripheral nerve damage [37]. This phenomenon, as well as the increase of neuronal receptive fields (i.e. the peripheral area that activates spinal neurons), might contribute to the neuronal basis of allodynia, hyperalgesia and spontaneous pain seen in NP. *Neuropeptide plasticity* is seen as a consequence of the phenotypic switch of primary afferents and it is characterized by an altered expression of neuropeptides and changes in their receptor numbers in the dorsal horn (Table 4) [38,39].

The *spinal reorganization* is a response to peripheral nerve injury of the Aβ-fibers (large myelinated afferents) that sprout into lamina II of the dorsal horn, which is normally innervated by C-fibers (small afferent fibers). The Aβ-fibers establish functional synaptic contact with second-order neurons [3]. As a consequence of these new synapses, low-threshold non-noxious inputs from the Aβ-fibers can be interpreted as nociceptive in origin although they are not [40]. Furthermore, Aβ-fibers suffer a phenotypic switch and begin to express nociceptors, substance P and CGRP. Nociceptors are normally expressed by primary afferent C-fibers and Ad-fibers, although they might be down-regulated after peripheral nervy injury [41].

Table 4. Neuropeptides with Altered Expression in Neuropathic Pain Models

Substance P
Calcitonin gene-related peptide (CGRP)
Galanin
Vasoactive intestinal polypeptide (VIP)
Adenylate cyclase-activating peptide (PACAP)
Neuropeptide Y (NPY)
Cholecystokinin (CCK)
Somatostatin
Melanocortin

Changes in inhibitory pathways, i.e. reduction in the amount of inhibitory control over dorsal horn neurons through different mechanisms are also important [14,42,43]. Since the emergance of the gate control theory of Wall and Melzack [44], the existence of an inhibitory system at the spinal cord may impede the transmission of low-threshold stimuli is known. Therefore, the reduction in neuronal inhibition will be followed by an enhancement of the nerve transmission of nociceptive inputs, thus giving an increase in pain sensations. The main question is what neurotransmitters are mainly responsible for such inhibitory effects. Until now, most attention has been focused on gamma-aminobutyric acid (GABA), the most abundant inhibitory neurotransmitter in the CNS, and also on glycine. This is the neurochemical basis of using gabergic agents that can increase the inhibitory tone in the spinal cord. Several lines of evidence suggest that there is a tonic gabergic and glycinergic inhibition of low-threshold afferents that innervate mechanoreceptors in non-injured rats [19]. As a consequence, reduced activity of inhibitory neurons may produce mechanical allodynia. In fact, a reduction in GABA immunoreactivity and the number of GABA immunoreactive cells in the spinal cord has been demonstrated after nerve injury [45,46]. The possibility of improving GABA activity has been suggested by Kontinen and Dickenson [47], who have shown that midazolam may reduce C-fiber-evoked firing in neuropathic rats but not in control animals.

Some of these mechanisms have been targeted to investigate new drugs that may be used in the treatment of NP. In the present review, we will comment on several of them. An in-depth review of some of these targets is considered in other papers of the current issue.

ON THE LIMITATIONS OF CURRENTLY AVAILABLE TREATMENTS

In the last fifty years, the treatment of NP has included antidepressants, antiepileptics, anticonvulsants, antiarrhythmics, topical local anesthetics, capsaicin and, not without controversy, opioids [Table 5]. We will summarize the evidence of their clinical effectiveness but it is generally

accepted that only a subset of patients significantly improve after receiving such drugs. Hence, there is an urgent need for more effective and adequate drugs.

Table 5. Main Pharmacological Treatments That are Currently Being Used in Neuropathic Pain

Antidepressants (amitriptyline, maprotiline, SSRIs)
Antiepileptics (gabapentin, carbamazepine, clonazepam, lamotrigine, topiramate, phenytoin)
Local anesthetics and antiarrhytmics (mexiletine)
GABA agonists (baclofen)
Opioids (morphine, tramadol)
Drugs acting on NMDA receptors (dextrorphan, ketamine)
Drugs acting on noradrenergic pathways (guanetidine, clonidine)
Capsaicin

The efficacy of antidepressants in NP state is beyond any doubt (see Sindrup and Jensen [48] for a review). However, it must be considered that only those with a tricyclic structure have demonstrated its usefulness, whereas the serotonin selective reuptake inhibitors (SSRIs) are far from being so good as they are in depression. Since the first suggestion of their analgesic effect in 1960, tricyclic antidepressants have become one of the first line drugs used in different NP conditions. Several authors have reviewed the available clinical evidence of their efficacy in controlled clinical trials [49,50]. The main conclusion that can be drawn is that the tricyclics may alleviate 60-70% of patients with NP in a wide range of conditions, such as painful neuropathy, post therapeutic neuralgia, and central post-stroke pain or nerve-injury pain. These drugs should not be used in patients with some cardiac conduction disturbances, congestive heart failure or convulsive disorders. Side effects are frequent, especially with amitryptiline, and may preclude its use, as the analgesia is not compensated for the severe dry mouth or the dizziness that some patients experience.

Anticonvulsants have been increasingly used to treat NP since they showed to be effective almost four decades ago [51]. Since then several anticonvulsants, such as carbamacepine, phenytoin and, more recently, gabapentin and lamotrigine, are routinely used to treat different NP conditions. Although only few clinical trials have been performed with these drugs, several of them have established the effectiveness of carbamazepine and gabapentin [52]. For instance, the former has shown itself to provide pain relief to 70%-89% of patients with trigeminal neuralgia after 1-2 weeks of treatment [51,53,54]. However, its efficacy against other NP conditions has not been so clearly established. Moreover, up to 50% of patients experienced side effects and up to 10% withdrew the treatment because of these [51]. Gabapentin is the first anticonvulsant that has been systematically studied in NP. This drug has been shown to relieve patients with painful diabetic neuropathy [55] and PHN [56], and its effectiveness is similar as observed with amitriptyline but with fewer side effects [56]. Dizziness and somnolence are the most frequent adverse effects but are generally well tolerated. Gabapentin has no direct action on GABA transmission and it seems to act on the $\alpha_2\delta$ subunit of calcium channels [57]. Pregabalin, an analog of gabapentin, is being developed as a new drug to treat NP conditions and has shown to provide analgesic relief [58]. The term 'gabapentinoids' is used to define this new family of drugs.

Systemic local anesthetics, such as lidocaine, have been used to treat NP. These drugs would act by blocking spontaneous ectopic activity in peripheral nerves and dorsal root ganglion, but postsynaptic actions on the NMDA receptor has been suggested [59]. Surprisingly, the effect of single infusion of lidocaine has prolonged the relief and this effect merits to be explored. In contrast, mexiletine has not shown consistent analgesic effects on NP in most studies [52].

Topical agents for NP include local anesthetics and capsaicin. The latter is useful but has significant limitations derived from its ability to activate then block, vanilloid receptors. As a consequence, capsaicin causes a severe burning sensation in some patients who refrain from its use for this reason. This limitation may be overcome if drugs that blocked vanilloid receptors lacked agonist activity (see next section). The best-studied drugs have been topical anesthetics, mostly lidocaine alone or combined with prilocaine [60]. The rationale behind the use of these drugs is the blockade of peripheral factors, like ectopic discharges may be sensitive to lower concentrations of local anesthetics than needed to block intact nerves [61]. Several studies have shown that 50%-80% of patients with posttherapeutic neuralgia may obtain partial relief with lidocaine patches with minimal side effects [60]. This treatment may be used alone or as an adjunct to oral agents to improve pain control.

Finally, a few words regarding the use of opioids in NP. Until recently, it has been dogmatic in pain medicine that NP had been resistant to the administration of opioid drugs [62]. However, several controlled clinical trials have shown that this painful condition may also improve after the administration of morphine (63], fentanyl [64], oxycodone [65] or tramadol [66]. Even when the analgesic effects with the last two are modest, these data suggest that the assumption that opioids are useless in NP is no longer acceptable and opens a new research front to test old and new opioid drugs in this condition.

TOWARDS A NEW PHARMACOTHERAPY OF NEUROPATHIC PAIN

In the last ten years, data obtained from research in many laboratories have opened new perspectives for finding drugs that may act by previously unknown mechanisms. In the next paragraphs, some of them will be considered.

Vanilloid Receptors

The vanilloid receptor or VR1 was discovered several years ago by Caterina *et al.* [67] as the molecular target where capsaicin acts to produce its known burning and analgesic effects. This receptor is an excitatory ion channel expressed by nociceptors and plays an important role in the detection and integration of pain following thermal or chemical stimuli. Capsaicin is an agonist of such receptors, and its analgesic effect is a consequence of repeated activation that finally desensitizes them. The effects that

follow this mechanism of action are predictable: an initial intense, burning sensation (as the receptor is activated), and a final analgesic effect (as the receptor is desensitized). This dual effect is poorly tolerated by some patients and, hence, new drugs that do not stimulate the VR1 but still have the blocking action are needed. Some compounds, like olvanil (NE-19550) or nuvanil (NE-21610), have analgesic and anti-inflammatory effects, may be given by oral route but cause severe hypothermia [68]. Nuvanil has less agonist activity on VR1 than capsaicin and inhibits hyperalgesia and allodynia induced by burns [69]. Resiniferatoxin, a compound from the cactus-like plant *Euphorbia resinifera*, has strong antagonistic properties and has shown to be useful in experimental models [70]. Arginine-rich peptides have also shown to be effective in blocking VR1 and behave as analgesics in pains induced by ocular application of capsaicin [71]. These evidences suggest that drugs acting on VR1 may be considered as candidates to be evaluated in NP [72]. Recent results show that the VR1 antagonist capsazepine reverses mechanical hyperalgesia in the partial sciatic nerve ligation model [73,74].

Tetrodotoxin-Resistant Sodium Channels (TTX-R)

In recent years, it has been observed that damaged neurons may express a distinct type of Na channels with unusual pharmacology, and they are especially attractive as pharmacological targets to produce analgesia. Some of these channels are resistant to tetrodotoxin, a well-known blocker of 'traditional' sodium channels, and are especially abundant in dorsal root ganglia. Their different pharmacological and biophysical properties and also their localization in pain pathways make them notably interesting in NP. Two of these channels, named SNS/PN3 (or $Na_V1.8$) and NaN/SNS2 ($Na_V1.9$) have been studied in experimental pain states [75]. In the case of NP models, it has been shown that interfering with SNS/PN3 expression by using antisense oligodeoxy-nucleotides prevents hyperalgesia and allodynia caused by chronic nerve injury [76]. However, no effect was seen when acting on the NaN/SNS2 protein. Given the restricted distribution of PN3 to sensory neurons, it confirms the possibility of using such channels as a pharmacological target, even when subtype specific sodium channel blockers are still not available despite the considerable efforts [75]. Recently, NW-109, a compound that blocks both TTX sensitive and resistant (TTX-R) channels, has shown its anti-allodynic effects in experimental NP when given orally and the effective dose is much lower than is needed to cause neurological deficits [77]. This study suggests the possibility of separating the activities in both channels even if the *in vitro* experiments show no specificity for TTX-R channels.

Calcium Channels

It is well known that the release of the neurotransmitter is mediated by calcium entry via calcium channels in the presynaptic neuron. Hence, the inhibition of substance P or glutamate release at nociceptive pathways by interfering with calcium entry would result in an analgesic effect [78]. However, neuronal calcium channels are so ubiquitous that this type of specific blockade is difficult to achieve. However, several lines of evidence seem to indicate that the interference of calcium channels may be used in the treatment of NP. For instance, the conopeptide ziconotide (SNX-11), a powerful blocker of N-type calcium channels, has been shown to be clinically useful in patients with several types of NP [79,80]. The usefulness of ziconotide is, however, limited by the need of using intrathecal route of administration, but these studies have shown that it is feasible to block N-type calcium channels to obtain pain relief.

A second proof of this pharmacological target is the observation that gabapentin also blocks the N-type calcium channel current at dorsal root ganglion neurons [81]. This drug has repeatedly shown its usefulness in relieving NP in experimental models and in patients [82]. Nonetheless, its mechanism of action was elusive as the presumed activity on GABA neurons did not explain its analgesic effects and the specific binding to the $\alpha_2\delta$ subunit of calcium channels was only a speculative mechanism. However, recent experimental evidence suggest that drugs acting on this subunit may constitute a new family of atypical analgesic drugs [83,84].

NMDA Receptors

As stated above, NMDA receptors are especially important in the induction and maintenance of NP. The blockade of its activation has been tested using different strategies, such as competitive antagonists, blockers of strychnine-insensitive glycine site ($glycine_B$), polyamine site (NR2B selective) or phencyclidine site located in the cationic channel [85]. Many of the studies devoted to showing the analgesic effects of this target in NP have been successful [86]. Even some of the compounds, such as ketamine, have been shown to be analgesic in clinical NP, but their use is limited by the frequency and severity of side effects. Additionally, the physiological activation of NMDA must be allowed while the pathological stimulation is prevented. This is a challenging situation that has been dealt with using different strategies. It has been shown that moderate affinity channels blockers, $glycine_B$ site antagonists or NR2B antagonists show a much better profile than the high affinity channel blockers and competitive NMDA receptor antagonists [85]. Moreover, the discovery that peripheral NMDA receptors may also be involved in inflammatory and visceral nociception merits further studies to analyze if these compounds may also be useful in NP states. For instance, the dose of the $glycine_B$ antagonist MRZ 2/576 that reduces autonomic responses in the model of ureter distension [87] is 10-fold lower than that needed to block NMDA receptors in the central nervous system.

Cannabinoid Receptors

Cannabinoid compounds produce their pharmacological effects by acting on two types of G-protein-coupled receptors; CB_1 receptors, which are mainly expressed in the central nervous system, and CB_2 receptors that are abundant in cells of the immune system [88]. Several fatty acid derivatives have been identified as endogenous cannabinoid ligands, such as anandamide, 2-arachidonoylglycerol, noladin ether and virodhamide [89]. The activation of cannabinoid receptors by endogenous or exogenous ligands

mainly results in inhibitory effects on the cell [89]. This endogenous cannabinoid system seems to participate in the physiological regulation of pain and may therefore represent an interesting target for the development of new analgesic compounds. The presence of cannabinoid receptors in different peripheral and central structures related to the transmission of nociceptive messages as well as the analgesic properties exhibited by natural and synthetic cannabinoids in different animal models confirms this hypothesis [90-93].

Animal studies performed in different experimental models of NP have demonstrated a potent action of cannabinoids in alleviating the allodynia and hyperalgesia that characterize this chronic pain state. Thus, in the partial sciatic ligation model in the rat, the systemic administration of the cannabinoid agonists-WIN55,212-2, CP-55,940 and HU-210, was able to reverse the mechanical hyperalgesia [94]. Similarly, the intrathecal and peripheral administration of WIN55,212-2 were also effective through a CB_1 receptor-mediated mechanism [94]. WIN55,212-2 has also been shown to reduce thermal and mechanical hyperalgesia as well as mechanical allodynia in two different NP models: the spinal nerve ligation and the chronic constriction injury of the sciatic nerve [95,96]. This last study reported an increase in thermal hyperalgesia and mechanical allodynia by administration of the specific CB_1 antagonist, SR 141716A, indicating the possible existence of an endogenous cannabinoid tone to control this physiopathological process. In agreement with this hypothesis, an up-regulation of cannabinoid CB_1 receptor mRNA has been reported in the rat thalamus after peripheral nerve damage [97], as well as activation of CB_1 receptors through neuronal projections from the nucleus reticularis gigantocellularis [98]. This structure is located in the rostroventromedial medulla, and participates in the descending analgesic pathway.

CB_1 receptors are found in the dorsal horn of the spinal cord where they are located on intrinsic spinal neurons and nerve terminals of afferent sensory neurons [99]. However, only a small percentage of CB_1 receptors is present on unmyelinated small-caliber fibers (C fibers), currently responsible for pain transmission, whereas these receptors are abundant in axons of larger diameter neurons (Aδ- and Aβ-fiber neurons) [100]. This localization provides a neuroanatomical substrate for the efficacy of cannabinoids in the treatment of NP. Indeed, this disorder is associated with an aberrant pain transmission characterized by abnormal painful spontaneous discharges in these myelinated fibers [101,102]. By contrast to cannabinoid receptors, the majority of opioid receptors is located on C fibers, as demonstrated by the important loss of these receptors in the spinal cord of rats following neonatal capsaicin treatment [103], which could explain the reduced efficacy of morphine in the alleviation of NP. Although the involvement of CB_1 receptors has been well documented, some recent reports indicate that the activation of peripheral CB_2 receptors could also be useful to inhibit the allodynia and hyperalgesia produced in an NP model. Thus, the selective CB_2 agonist, AM1241, reverses tactile and thermal hypersensitivity produced by peripheral nerve injury in mice and this effect is preserved in CB_1-receptor knockout mice, demonstrating that this response is not mediated by CB_1 receptors [104,105]. This result is of great interest considering the absence of CB_2 receptors in the central nervous system. Therefore, the possible use of CB_2 agonists would be devoid of the psychotropic side effects that are exclusively due to the activation of central cannabinoid receptors.

In conclusion, the increasing knowledge of the neurophysiological and neurochemical bases of NP allows further insight in the discovery of new pharmacological targets. However, given the previous experience, a drug that modifies a pathophysiological mechanism does not imply that such a new type of drug will reach the market. Efficacy is a key issue, but long-term safety must be considered when the treatment of a chronic condition like NP is considered.

ABBREVIATIONS

IASP	=	International Association for the Study of Pain.
CB	=	Cannabinoid.
CCI	=	Chronic constriction injury.
CCK	=	Cholecystokinin
CGRP	=	Calcitonin gene-related peptide.
CNS	=	Central nervous system.
DRG	=	Dorsal root ganglia.
GABA	=	gamma-aminobutyric acid .
NGF	=	Nerve growth factor.
NMDA	=	N-methyl-D-aspartate.
PACAP	=	Adenylate cyclase-activating peptide .
PHN	=	Postherpetic neuralgia.
PLN	=	Partial nerve lesion.
SNL	=	Spinal nerve ligature.
SSRIs	=	Serotonin selective reuptake inhibitors.
TTX-R	=	Tetrodotoxin-resistant sodium channels.
TTX-S	=	Tetrodotoxin-sensitive sodium channels.
VIP	=	Vasoactive intestinal polypeptide

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