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Narrative Review

Pulsed Radiofrequency in Interventional Pain Management: Cellular and Molecular Mechanisms of Action – An Update and Review

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Background: Pulsed radiofrequency (PRF) treatment uses low energy, short pulsations to modulate tissue characteristics. PRF treatment has been effective as an interventional pain management technique to treat a variety of chronic neuropathic pain (neuralgia) disorders, but a comprehensive review of its biological mechanism has not been updated in a decade.

Objectives: In this literature review, we performed a literature search in PubMed to identify publications describing the mechanisms of action of pulsed radiofrequency for pain indications.

Study Design: Narrative literature review.

Methods: A systematic search was performed through PubMed from database inception to December 31, 2019, to identify all articles addressing the cellular or molecular mechanisms of action of PRF on neuropathic pain. The search terms "pulsed radiofrequency" and "pulsed radiofrequency mechanisms" were used. Cellular and molecular mechanisms of PRF interventions were subdivided into 3 broad categories: nociceptive signalling, immune activity, and synaptic function. A total of 20 publications were identified for inclusion in this updated review.

Results: It was found that pulsed radiofrequency impacts many different biological pathways involved in the modulation of chronic neuropathic pain (neuralgia). With regards to nociceptive signalling, PRF treatment modulates ion channels (Na/K ATPase, HCN, P2X3), CGRP, neurotransmitters (aspartate, citrulline, M-ENK, glutamate), postsynaptic receptors (AMPA-R, GABA-B), and synaptic function (KCC2). PRF treatment also modulates immune activity, including microglial markers (CD3, CD56, Iba1), inflammatory cytokines (IL-6, IL-17, IRF8, IFN-γ, TNFα), and intracellular proteins implicated in immune mediated neuropathic pain (BDNF, β-catenin, JNK, p38, ERK1/2).

Limitations: This review is primarily limited by the diverse data sets that needed to be collated and correlated, as no study was comprehensive in addressing all markers, cytokines, pathways, neurotransmitters, ion channels, proteins, genes, and gene expression changes, along with their clinical outcomes concurrently. As such, the interplay of these individual pathways and mechanisms and their isolated effects on efficacy of PRF cannot be concluded. Rather, the large majority of findings can be seen as associations instead of definitive causal relationships to clinical outcomes.

Conclusions: Herein describes a clinically relevant collated update describing the cellular and molecular mechanisms of action of PRF for pain management.

Key words: Biomarkers, chronic pain, cytokines, ganglia, hyperalgesia, immunomodulation, neuralgia, neurotransmitter, nociceptors, pulsed radiofrequency treatment, receptors, spinal

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Chronic pain affects approximately 7.6 million Canadians and approximately 116 million American adults living with chronic pain, more than the total number of patients affected by cancer, diabetes, and heart disease combined (1,2). Neuropathic pain, as opposed to nociceptive pain which is a result of actual or threatened damage to non-neural tissue and activation of nociceptors with normally functioning somatosensory nervous system, is defined as an injury or disease to the somatosensory system (3). Although the understanding of the pathophysiology of chronic neuropathic pain continues to evolve, many consider etiologies as split into peripheral and central causes of sensitization (4,5). Peripheral etiologies, alternatively termed peripheral sensitization, are thought to be nervous injuries that lead to spontaneous ectopic discharges and ectopic hyperexcitability (4). Hyperactivity of the peripheral nervous system, in addition to resulting in increased painful stimuli itself, is postulated to enhance nociceptive signalling and increase the production and release of a myriad of proinflammatory mediators and proinflammatory cytokines (4). The creation of this hyper-excitability and inflammatory milieu primes c-fibers in the central nervous system (CNS) and leads to increased CNS signalling, termed "Central Sensitization" (5). Conventional treatments are aimed toward blocking individual pathways and include the judicious use of simple analgesics and neuropathic pain medications, such as, gabapentinoids, tricyclic antidepressants, and serotonin norepinephrine reuptake inhibitors, in conjunction with nerve blocks, 'orthobiologic' injections, and spinal cord stimulation, as indicated (6,7).

Pulsed radiofrequency (PRF) is an interventional pain management technique that has been effective in the treatment of thoracic postherpetic neuralgia, trigeminal neuralgia, radicular pain, and many other indications (8-12). PRF delivers a low-energy electrical field in rapid pulsations to target nervous tissue and associated microglia. Compared to high-temperature radiofrequency ablation (RFA), PRF is not ablative, but instead neuromodulating (13-15). Although PRF has demonstrated efficacy in the treatment of neuropathic pain conditions and has itself been widely adopted, a comprehensive review of mechanisms of action has not been published in almost a decade despite significant breakthroughs in the understanding of the molecular and cellular effects of PRF (13).

Thus, the objective of this review is to identify, consolidate and update the known and proposed mechanisms of action of PRF with a specific focus on the cellular and molecular modulating effects and how they may reduce neuropathic pain.

Methods

Search Strategy

A literature search was performed through PubMed from database inception to December 31st, 2019, to identify all articles addressing the cellular or molecular mechanisms of action of PRF on neuropathic pain. The search terms "pulsed radiofrequency" and "pulsed radiofrequency mechanisms" were used. This combined search revealed 1,119 results. Articles that explored mechanisms of action for non-pain indications were excluded, as were nonoriginal articles that lacked novel data, or those that lacked controls or the appropriate statistical analyses. Clinical outcomes data for PRF was not included in this review as outcomes have been described previously (13,16). A total of 20 publications were identified for inclusion in this updated review. Cellular and molecular mechanisms of PRF interventions were subdivided into 3 broad categories: nociceptive signalling, immune activity, and synaptic function.

Results

Nociceptive Signalling

Neurotransmitters

Nociceptive receptors are found on specialized peripheral sensory neurons that are triggered in the advent of harmful stimuli. These harmful stimuli are propagated from the peripheral nervous system to the CNS by the action of endogenous chemical messengers (neurotransmitters) and then interpreted centrally as pain. One well-studied receptor involved in nociceptive signalling is α-amino-3-hydroxy-5-methy-4-isoxazole propionate (AMPA-R). AMPA-R is a ligand-gated ion channel composed of 4 homologous subunits, GluA1-GluA4, that is positioned in the postsynaptic neuronal membrane. This channel is involved in fast synaptic transmission of nociceptive signals by action of glutamate binding (17). In rodent radicular neuropathic pain models, PRF led to internalization of GluA1 and GluA2 subunits (Fig. 1) (18). This resulted in a lower synaptosome-cytosol ratio of GluA1/2 and long-term depression of nociceptive signalling (18). On the pre-

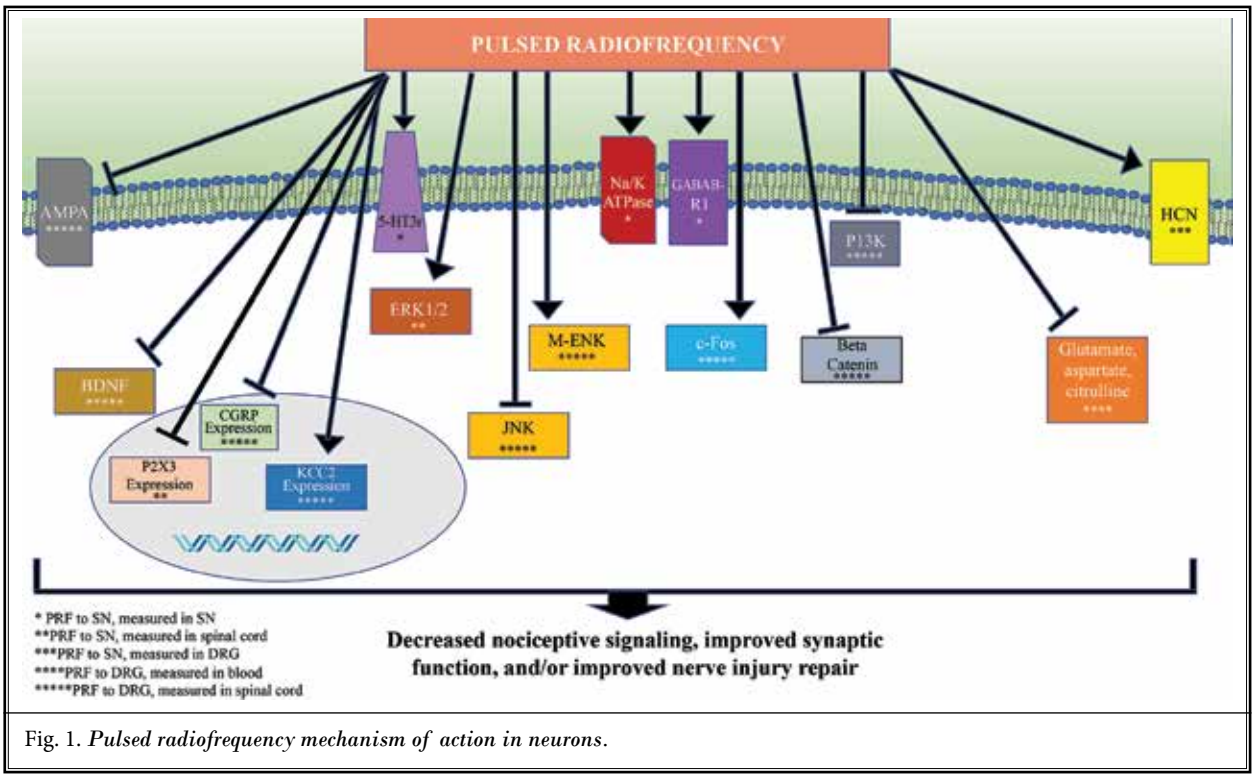

Fig. 1. *Pulsed radiofrequency mechanism of action in neurons.*

synaptic end, glutamate levels were attenuated following PRF treatment in diabetic neuropathic pain rodent models. Compared to sham treated rats, PRF treatment decreased heat hyperalgesia, mechanical hyperalgesia, and cold allodynia (19). Attenuation of aspartate and citrulline (nitric oxide release marker) is also seen, leading to relief of mechanical allodynia in complete Freund's adjuvant (CFA) inflammation induced rodents following PRF (20). Both glutamate and aspartate are excitatory amino acids (EAA) and their inhibition by PRF leads to decreased nerve cell polarization.

In addition to attenuation of nociceptive signal propagation, enhancing inhibition pathways is another mechanism of action for PRF. Gamma-aminobutyric acid B receptor 1 (GABA(B)-R1) and 5-hydroxytryptamine receptor 3a (5-HT3r) are G-protein coupled receptors and ligand-gated channels, respectively. GABA is an inhibitory neurotransmitter. Binding of GABA to its transmembrane receptor on the postsynaptic neuron hyperpolarizes the cell via calcium influx (21). In a similar manner, binding of serotonin to 5-HT3r induces hyperpolarization, and thus inhibits nociceptive signal propagation (22). Both of these inhibitory receptors are implicated in PRF's mechanism of action. RT-qPCR of spared nerve injury (SNI) rat models treated with PRF revealed an upregulation of GABA(B)-R1 and 5-HT3r compared to control. Upregulation of these inhibitory pathways by PRF treatment reversed mechanical allodynia in this model (23).

In addition, PRF induces upregulation of met-enkephalin (M-ENK) concentrations in a SNI-model (24). M-ENK is an endogenous opioid neurotransmitter that serves as an agonist for opioid receptors. Activation of these receptors by M-ENK reduces nociceptive stimulation at both pre- and post-synaptic neurons (25). M-ENK levels increased significantly following PRF treatment in SNI-models and their mechanical threshold increased, as well (24).

Together, the above evidence suggests PRF modulates both excitatory and inhibitory neurotransmitter activity to decrease nociceptive signalling in neuropathic pain models.

Ion Channels

Ion channels play a critical role in all signal propagation within the CNS and peripheral nervous system (PNS). The Na/K ATPase channel is widely distributed across many cell types and plays an important role for action potential propagation in nociceptive signalling (26). RT-qPCR of the dorsal root ganglion (DRG) and spinal cord in SNI rat models revealed downregulation of Na/K channels (23). This results in prolonged hyperac-

tive synaptic transmission of nociceptive signals resulting from impaired resting potential states in neurons. PRF treatment in these targeted sites provided an analgesic effect and reversed attenuation by upregulating gene expression of Na/K channels (23).

In addition to Na/K channels, evidence suggests hyperpolarization-activated cyclic nucleotide-gated (HCN) channels also play a role in PRF's mechanism of action. In the CNS and PNS, HCN channels are implicated with neuronal excitability, synaptic integration, and resting membrane potential formations. HCN regulates glutamate release via voltage-gated channels by modulating synaptic polarity (27). Chronic constriction injury (CCI) rat models have demonstrated downregulation of HCN1 and HCN2 subunits in neuropathic pain states (28). PRF treatment resulted in upregulation of HCN in the DRG compared to sham controls, which lead to reduced hyperalgesia and allodynia (28).

Purinergic ligand-gated ion channel 3 (P2X3) receptor also appears to be affected by PRF therapy. The P2X3 receptor is selectively expressed in afferent sensory neurons and is activated via ATP (4). Activation of this receptor leads to nociceptive signal propagation by calcium influx along with transmembrane movement of sodium and potassium. Using a rat CCI model, mRNA levels of P2X3 were found to be significantly reduced following PRF treatment to the sciatic nerve when compared to controls (29). This finding suggests PRF downregulates P2X3 expression to alleviate nociceptive signals as evidenced by improved allodynia and hyperalgesia.

Small Peptides

In addition to receptors and ion channels, short peptides are also implicated in pain signalling and modulated by PRF. Calcitonin-gene related product (CGRP) is a 37 amino acid peptide responsible for neuronal growth, survival, and directed differentiation. In regards to pain sensitization, CGRP binding to its receptor triggers downstream activation of protein kinase A (PKA) and protein kinase C (PKC) which, ultimately, result in AMPA-R activation via phosphorylation, thereby resulting in calcium influx into the cell lowering the action potential threshold (4). Immunohistological data from lumbar disc herniation rodent models reveals PRF to have a role in modulating CGRP, noting that expression was decreased following treatment compared to sham PRF procedures (30,31). Also, mechanical withdrawal thresholds increased, suggesting attenuation of nociceptive signalling.

Immune Activity

Microglial Activity

Pathologic microglia proliferation and activation is implicated in the onset and propagation of neuropathic pain (4). Multiple surface markers serve as a proxy for microglial activity including ionized calcium binding adaptor (Iba1). In a lumbar disc herniation model, caudal epidural PRF reduced levels of Iba1 (30). Similar trends have been demonstrated in human radicular pain cohorts where PRF treatment resulted in decreased CD56 and CD3 levels, cell surface receptors that are markers of microglia activity due to their responsibility in cell adhesion, synaptic plasticity, and T-cell activation (Fig. 2) (32).

Inflammatory Cytokines

Pathologic inflammation has been well-documented as a cause of neuropathic pain (5). Lesions to the somatosensory system may be due to aberrant auto-immune reactivity, leading to the onset, or maintenance, of neuropathic pain. Interleukin 6 (IL-6) and interleukin 17 (IL-17) have been reported to correlate or inversely correlate, respectively, with neuropathic pain and their levels have been shown to be modulated by PRF (5,33,34). IL-6 partakes in JAK/STAT pathways in microglia to trigger tissue inflammation (35). In radicular pain patients, post-PRF treatment measurements identified lower levels of IL-6, which appears to be due to alterations in expression of IL-6 within the DRG (36). IL-17 levels were shown to inversely correlate with chemotherapy-associated neuropathic pain, which is upregulated after treatment with PRF (33,34). PRF's ability to modulate IL-17 levels gives insight into its underlying mechanism in neuropathic pain (36).

In addition to interleukins, interferon gamma (IFN-γ) is another inflammatory molecule involved in the adaptive and innate immune system. Similar to IL-6, IFN-γ binding to its receptor triggers the JAK/STAT pathways to activate microglia. IFN-γ levels have been correlated to neuropathic pain and growing supporting evidence signifies its role in increasing expression of Iba1 and P2XR (37). In the abovementioned cohort of radicular pain patients, PRF significantly reduced IFN-γ and induced analgesia (36).

Other interferon subtypes may be implicated in inflammation-induced neuropathic pain through interferon regulatory factor 8 (IFR8). IFR8 is a transcriptional regulator of IFN-α and IFN-β, as well as other genes involved in the development of neuropathic pain (38). Al-

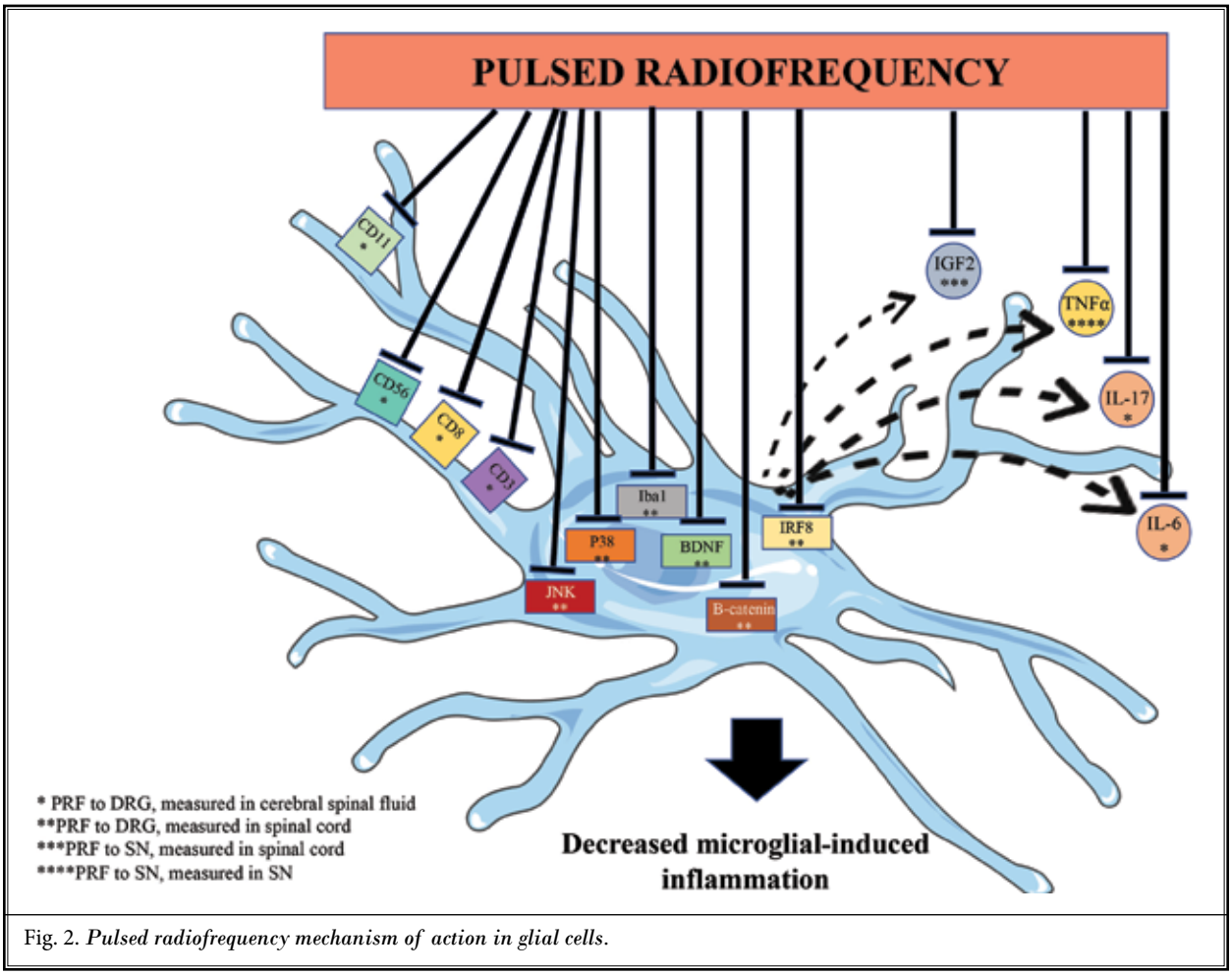

Fig. 2. *Pulsed radiofrequency mechanism of action in glial cells.*

though there is no current evidence of PRF modulating IFN-α and IFN-β, it does appear to affect IRF8 activity. PRF on the DRG in SNI-rats attenuated IFR8 levels and improved mechanical allodynia, which is a commonly reported outcome of IRF8 hyperactivity (38-40).

Tumor necrosis factor alpha (TNFα) is a cytokine widely distributed across the CNS and PNS. TNFα is upregulated states of increased inflammation, including sepsis and nervous injury, and is responsible for triggering caspases, which leads to apoptosis of neuronal cells resulting in increased neuropathic pain (41). Levels of TNFα is significantly upregulated in both DRG and sciatic nerve of CCI models. Treatment of these models with PRF not only alleviated mechanical allodynia and thermal hyperalgesia, but also decreased TNFα levels in both DRG and sciatic nerve (42,43). Evidence suggests that part of PRF's mechanism of action is by downregulating key inflammatory cytokines including TNFα.

Aside from inflammatory cytokines, insulin-like growth factor 2 (IGF-2) has also been correlated with neuropathic pain. IGF-2 is an imprinted gene responsible for cellular proliferation, growth, and survival. In neurons, it has been reported to trigger inflammatory pathways via ERK1/2 pathways (43). SNI-models had elevated levels of IGF-2 compared to control. Treatment of PRF reversed aberrant IGF-2 expression to relieve neuropathic pain. Interestingly, PRF has also shown to inhibit ERK1/2 activation following SNI in this pathway, resulting in greater reduction in allodynia in treatment groups compared to controls (43).

Intracellular Proteins

Beta catenin (β-catenin) is an intracellular protein that plays a key role in the canonical/non-canonical WNT signalling pathway, which leads to the downstream transcription of inflammatory cytokines IL-18, TNFα, and glutamate receptors in the spinal cord (44). In CCI models, β-catenin levels were elevated, identi-

fying a correlation with neuropathic pain. β-catenin levels decreased significantly following PRF treatment to the DRG and sciatic nerve compared to sham (45).

PRF also modulates c-Jun N-terminal kinases (JNK) to reduce neuroinflammation in the DRG. Similar to β-catenin, JNKs trigger gene expression to regulate neuronal plasticity and inflammation. Translocation of activated JNKs into the nucleus leads to transcription of TNFα and IL-1B (46). PRF appears to inhibit JNK in CFA-induced hyperalgesia models, correlating with improvements in mechanical hyperalgesia (47).

The mitogen activated kinase (MAPK) pathway is another well-studied pathway that is implicated in neuropathic pain and modulated by PRF. P-p38 is the phosphorylated form of p38, which is a type of MAPK. This kinase's aberrant activity results in hypersensitivity after nerve injury by evoking P2X4R and brain-derived neurotrophic factors (BDNF) activity from microglia (4). Following PRF treatment in the DRG of CCI-induced rodents, microglial activity and p-p38 levels decreased in the spinal cord (40).

Furthermore, BDNF and its upstream activator phosphoinositide-3 kinase (PI3K) was also found to be modulated by PRF. BDNF has roles in inducing neural hypersensitivity, in addition to nerve growth and inflammation-induced pain. BDNF binding to TrkB receptors triggers downstream increases in intracellular chloride, suppression of GABA-A inhibition, and thus depolarization with subsequent allodynia (5). PRF to the DRG inhibited SNI-induced BDNF upregulation and partially reversed allodynia in rodents (48).

Synaptic Function

As previously discussed, GABA plays an inhibitory role in nociceptive signalling and PRF acts to directly enhance this inhibition, but PRF may also enhance nociceptive inhibition by altering epigenetic profiles of nerve cells. In CFA rodents, it was found that histone subunits H3/H4 are hypoacetylated, resulting in decreased expression of K-Cl-Cotransporter-2 (KCC2) (49). KCC2 maintains intracellular chloride gradients, which is essential for GABA-ergic and glycinergic transmissions (5). PRF in the DRG appears to restore histone acetylation of H3/H4 and increase expression of KCC2 to enhance GABA-mediated nociceptive inhibition, resulting in attenuation of sensitized pain behaviour in these rodents (49).

Discussion

PRF is an interventional pain management technique used for treating chronic neuropathic pain. There are several studies that evaluated and proved the efficacy of PRF in treating different pain conditions. The exact mechanism of action is still unclear.

Herein, we provided a comprehensive literature review evaluating the cellular and molecular mechanisms underlying PRF mechanisms of action for modulating and reducing chronic neuropathic pain. PRF modulates many different pathways involved in nociceptive signaling, immune activity, and synaptic function, which, individually and in concert, are thought to pathologically result in chronic neuropathic pain.

This review is primarily limited by the diverse data sets that needed to be collated and correlated, as no study was comprehensive in addressing all markers, cytokines, pathways, neurotransmitters, ion channels, proteins, genes, and gene expression changes, along with their clinical outcomes concurrently. As such, the interplay of these individual pathways and mechanisms and their isolated effects on efficacy of PRF cannot be concluded. Rather, the large majority of findings can be seen as associations instead of definitive causal relationships to clinical outcomes. Furthermore, limitations of individual studies also carry through to this review. Moreover, not all PRF is the same. With different parameters (frequency, pulse width, temperature, time, cannula and active tip size) variably utilized from study to study, it is possible that some of the tissue effects and mechanisms of action varied with changes in parameter, not only based on parameters as a whole, but for different sets of parameters in different tissue types (i.e., sympathetic ganglia, peripheral nerves, DRG) and different species (i.e., humans versus rodents). The multitude of potential parameters and their effects on different nerve tissue types remains to be studied to further subclassify the mechanism of action for various sets of PRF parameters. Lastly, the majority of studies were performed in rodents which, while useful in the identification and evaluation of cellular and molecular mechanisms, may or may not be translatable to human neuropathic pain pathophysiology and the human response to PRF therapy. Further investigation is warranted to fully elucidate the direct mechanisms of action of PRF for treatment of neuropathic pain in humans.

Conclusion

While clinical outcomes and indications for PRF have been described previously, this review describes cellular and molecular mechanisms of action of PRF as

an update to the known literature. PRF continues to be an increasingly popular interventional pain management technique used for treating chronic neuropathic pain with a growing body of cellular and molecular data describing its mechanisms of action.

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