TITLE

Therapeutic Approaches to Nociceptive Pain Based on Findings in the Reserpine-Induced Fibromyalgia-Like Animal Model

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RUNNING TITLE

Potential Therapeutic Approaches to Nociplastic Pain

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ABBREVIATIONS: AMPA, α -3-hydroxy-5-methyl-4-isoxazole propionic acid; ASIC, acid-sensing ion channel; ATP, adenosine triphosphate; AT-RvD1, aspirin-triggered resolvin D1; AT-RvD2, aspirin-triggered resolvin D2; BBG, brilliant blue G; 2-BFI, 2-(2-Benzofuranyl)-2-imidazoline; CaMKII, calmodulin-dependent protein kinase II; CAT, catalase; CNS, central nervous system; CoQ10, coenzyme Q10; CSF, cerebrospinal fluid; Cox, cyclooxygenase; DA, dopamine; DALBk, des-Arg⁹-[Leu⁸]-bradykinin; DRG, dorsal root of ganglia; EEG, electroencephalography; EPM, elevated plus maze; EZM, elevated zero maze; FM, fibromyalgia; FST, forced swimming test; GSH, glutathione; 5-HT, 5-hydroxytryptamine (serotonin); FAAH, fatty acid amide hydrolase; GABA, γ -aminobutyric acid; GluN2B, NMDA-type glutamate receptor subunit 2B; 11 β -HA, hyperalgesia; HS, hypersensitivity; HSD, 11 β -hydroxysteroid dehydrogenase; IASP, the International Association for the Study of Pain; IL, interleukin; iNOS, inducible nitric oxide synthase; KCC2, K⁺-Cl⁻ co-transporter 2; LA, locomotor activity; LPO, lipid peroxide; MA, mechanical allodynia; MAO, monoamine oxidase; (m-CF₃-PhSe)₂, m-trifluoromethyl-diphenyl diselenide; MDA, malondialdehyde; Mfn, mitofusin; MWM, Morris water maze; NA, noradrenaline; Nav, voltage-dependent Na⁺ channel; NF- κ B, nuclear factor- κ B; NGF, nerve growth factor; NLRP, nucleotide-binding oligomerization domain (NOD)-like receptor protein;

NMDA, N-methyl-D-aspartate; N/OFQ, nociceptin/orphanin FQ; NO, nitric oxide; NOP-R, nociceptin opioid receptor; NSFT, novelty-suppressed feeding test; OFT, open field test; PAM, positive allosteric modulator; PARP, poly ADP-ribose polymerase; PAT, passive avoidance test; PGC, peroxisome proliferator activated receptor gamma coactivator; PPAR, peroxisome proliferator-activated receptor; PSAP, p,p'-methoxyl-diphenyl diselenide, α - (phenylselenanyl) acetophenone; PSD-95, postsynaptic density protein 95; R, receptor; RIM, reserpine-induced myalgia; ROI, reactive oxygen intermediates; ROS, reactive oxygen species; SDH, superficial dorsal horn; SNRI, serotonin/noradrenaline reuptake inhibitor; SOD, superoxide dismutase; SSRI, selective serotonin reuptake inhibitors; TBARS, thiobarbituric acid reactive substance; TNF, tumor necrosis factor; TRPV1, transient receptor potential vanilloid type 1; TST, tail suspension test; VDCC, voltage-dependent calcium channel; VEGF, vascular endothelial growth factor

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ABSTRACT

Nociplastic pain, the third category of chronic pain, has emerged as a serious medical issue. Due to its significant negative influences on patients and society, high prevalence, and lack of sufficiently effective treatments, more efficacious therapies are required. This review highlights the potential therapeutic approaches identified in studies that used reserpine-induced myalgia (RIM) animal model that exhibits nociplastic pain-associated phenotypes. These studies have revealed that biological processes including the chronic reduction of monoamines, increase of oxidative/nitrosative stresses and inflammatory mediators, upregulation of pronociceptive neurotransmitters and their receptors, increase of trophic factors, enhancement of the apoptotic pathway, sensory nerve sensitization, and activation of immune cells in central and/or peripheral regions, underly the nociplastic pain-associated phenotypes in RIM animal model. Potential therapeutic approaches to nociplastic pain, i.e., 1) functional modification of specific molecules which expression is distinctly altered following monoamine reduction, 2) targeting the molecules which are responsible for other major categories of chronic pain (i.e., chronic inflammatory pain and neuropathic pain), 3) supplementation of nutrition to correct the disrupted nutritional balance, 4) improvement of physical constitution by natural substances, and 5) nonpharmacological interventions, have been identified.

SIGNIFICANCE STATEMENT

Studies in RIM animal model have revealed the pathologies that occur after the chronic reduction of monoamines and identified potential therapeutic approaches to nociplastic pain. Translation of their analgesic efficacy from RIM animal model to patients remains an issue to be addressed. Successful translation would lead to better therapies for nociplastic pain.

Introduction

Nociplastic pain has been recently defined by the International Association for the Study of Pain (IASP) as “pain that arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors or evidence for disease or lesion of the somatosensory system causing the pain” (IASP, 2017). This official definition has clarified that this pain is the third category of chronic pain and a crucial research subject along with other major categories of chronic pain (i.e., chronic inflammatory pain and neuropathic pain) (Woolf, 2010; Fitzcharles et al., 2021). Nociplastic pain accompanies a diverse range of clinical conditions including fibromyalgia (FM), chronic low back pain with unknown causes (non-specific low back pain with emotional distress and interference in daily activities), chronic temporomandibular pain (myogenous or arthrogeous pain in orofacial regions), and irritable bowel syndrome (abdominal pain associated with bowel habit alterations) (Fitzcharles et al., 2021). Given that nociplastic pain characterizes across the above-mentioned conditions, it would be an efficient approach to gain insights regarding potential therapies for nociplastic pain by focusing on one of those conditions and extrapolate the obtained insights to other conditions. This review, thus, focuses on FM, a well-studied representative disorder which presents with archetypal

nociplastic pain as a core symptom (Wolfe et al., 2010; Fitzcharles et al., 2021). FM is highly prevalent being the third most frequent musculoskeletal disorder that affects 2-3% of the general population worldwide (Sarzi-Puttini et al., 2020). The inexplicable widespread pain causes persistent bouts of discomfort in patients (Sarzi-Puttini et al., 2012). It has been suggested that alteration of pain processing at central and peripheral level is responsible for nociplastic pain in patients with FM (Sluka and Clauw, 2016; Siracusa et al., 2021). Because currently available therapeutics do not provide adequate relief of nociplastic pain in patients with FM (Häuser et al., 2014), more effective treatments are required (Nagakura, 2015).

A pivotal step in developing therapeutic approaches to nociplastic pain for preclinical research is to construct an animal model which simulates patients as a research tool. FM is at the forefront of the nociplastic pain-associated clinical conditions in terms of availability of relevant animal models. While carrageenan-induced inflammation in connective tissues of the lower back in rats (Corey et al., 2012) and chronic water avoidance stress-induced visceral pain in rats (Myers and Greenwood-Van Meerveld, 2012) have been used as models of non-specific low back pain and irritable bowel syndrome, respectively, several animal models simulating FM such as reserpine-induced myalgia (RIM) animal model (Nagakura et al., 2009), repeated intramuscular

acidic saline injection model (Sluka et al., 2003), and intermittent cold stress model (Nishiyori and Ueda, 2008), have been developed and utilized for studying symptoms, pathophysiology, and therapeutic approaches. A recent review article, which examined such currently available animal models of FM, demonstrates that RIM animal model simulates FM the most in terms of (1) exhibition of spontaneous and evoked pain phenotypes, (2) appearance of prevalent comorbidities such as depression, fatigue, and sleep disorders, (3) reduction of monoamines in the central nervous system (CNS) regions reflecting the hypothetical pathogenesis, and (4) similarities in sensitivities to existing pharmaceutical therapies (da Silva Brum et al., 2021). This review, thus, focuses on the evidence regarding the pathophysiology and potential therapeutic approaches to nociplastic pain, which has been identified in the studies using RIM animal model.

Outline of RIM Animal Model

Hypothesis for Development of Nociplastic Pain in RIM Animal Model

Accumulating evidence in patients with FM suggests that monoaminergic control disruption is involved in the pathogenesis of FM (Russell et al., 1992; Wood et al.,

2009; Ablin and Buskila, 2015; Macian et al., 2015). For example, diffuse noxious inhibitory control, a pain inhibitory modulation system mediated by descending serotonergic-noradrenergic neuronal pathways in the CNS, is deficient (Staud et al., 2003; Julien et al., 2005). The level of major metabolites of serotonin (5-HT) and noradrenaline (NA) is lowered in the cerebrospinal fluid (CSF) (Russell et al., 1992). Dysfunction of dopaminergic neurotransmission in the CNS is detected by brain imaging studies (Chew et al., 2019). The highly prevalent accompaniment of depression (Hirschfeld, 2000) and sleep disturbances (Scammell et al., 2017) infers the disruption of monoaminergic control in the CNS as a common origin of the nociplastic pain and such comorbidities. The analgesic efficacy of 5-HT/NA reuptake inhibitors (SNRIs) also supports the involvement of the disruption of monoaminergic control.

RIM animal model was constructed by building on the hypothesis that monoaminergic control disruption in the CNS would lead to the exhibition of nociplastic pain-associated phenotypes and comorbidities mimicking FM. Repeated administration of reserpine, a depletor of endogenous monoamines, simulates the disruption of monoaminergic control in the CNS, causes a marked decrease in the amount of dopamine (DA), NA, and 5-HT in brain regions, and induces muscle hyperalgesia (HA) and mechanical allodynia (MA), which persist for two weeks or

longer without any apparent organic abnormalities, mimicking nociplastic pain in patients with FM (Nagakura et al., 2009). Although reserpine has been used as an antihypertensive drug in the clinical setting, it does not usually cause chronic pain in humans (Webster and Koch, 1996). This discrepancy in the algescic effect of reserpine between humans and animals could be due to the different doses used. The therapeutic dose of reserpine in humans, 0.25 mg/day (around 0.003 mg/kg), is translated to 0.019 mg/kg in rats by the body surface area normalization method (Reagan-Shaw et al., 2008), which is about 50 times less than the dose of 1 mg/kg for inducing an FM-like condition in rats (Nagakura et al., 2009).

Protocol for RIM-Induced Nociplastic Pain-Associated Phenotypes

The animal species used for RIM animal model have been limited to rats and mice. In the case of rats, the mostly used reserpine application protocol is subcutaneous administration at 1 mg/kg once daily for three consecutive days (Nagakura et al., 2009). The subcutaneous route is preferred based on a study that demonstrated that a prolonged depletion of NA is achieved more steadily by subcutaneous than intraperitoneal administration of reserpine in rats (Martínez-Olivares et al., 2006). In the case of mice, there is a range of protocols used. Reserpine is preferably administered subcutaneously

at 0.25 (de Souza et al., 2014; Peres Klein et al., 2016; Nagakura et al., 2018), 0.5 (Kaur et al., 2021; Schossler Garcia et al., 2021a), or 1 mg/kg (Xu et al., 2013; Liu et al., 2014; Brum et al., 2020) once daily for three consecutive days. Intraperitoneal administration at 0.5 mg/kg once daily for three consecutive days is also employed (Sousa et al., 2018).

Evoked Pain Measurement in RIM Animal Model

RIM animal model manifests widespread (i.e., both sides of the body) and long-lasting (i.e., two weeks or longer) pain-associated phenotypes without apparent tissue damage, mimicking the clinical features of nociplastic pain. The most studied phenotype is the reduced threshold for response to externally applied stimuli, i.e., the evoked pain phenotype. MA (decreased threshold for withdrawal response to tactile stimuli in plantar surface), muscle HA (decreased threshold for withdrawal response to pressure stimuli onto the gastrocnemius muscle), and mechanical HA (decreased threshold for withdrawal response to pressure stimuli onto the paw) have been demonstrated (da Silva Brum et al., 2021). These phenotypes might be associated with the clinical features that tenderness to palpation is a former diagnostic criterion for FM, and that blunt pressure stimulation is used to estimate muscular pain thresholds (Petzke

et al., 2003). Enhanced sensitivity to temperature alteration stimuli is also demonstrated in animals. Heat HA has been detected by various methodologies such as the plantar test, hot plate test, and tail-flick test. Hypersensitivity (HS) to cold stimuli is also demonstrated by the acetone application test (da Silva Brum et al., 2021). Although the above-mentioned evoked pain phenotypes in RIM animal model may correspond to HS in the exposure of patients to external stimuli (e.g., pinprick HA) (Rehm et al., 2021), they do not fully reflect the primary symptom characterized by spontaneous ongoing pain in patients with FM.

Alteration of Spontaneous Behavior and Physical Function in RIM Animal Model

The endpoint measures for the assessment of pain in animal models should simulate the primary sign, i.e., spontaneous ongoing pain, in patients with chronic pain (Percie du Sert and Rice, 2014; Tappe-Theodor and Kuner, 2014; Nagakura, 2017; González-Cano et al., 2020). Recently, spontaneous behaviors and physical functions, which are significantly affected by the existence of chronic pain, have been measured in animal models, because they possibly reflect the spontaneous ongoing pain in patients (González-Cano et al., 2020). Experiments conducted so far have demonstrated that alterations of spontaneous behaviors and physical functions occur in RIM animal model

as follows. Naturally occurring behaviors such as burrowing (Brusco et al., 2019), wheel running (Favero et al., 2017), and exploratory locomotion (Pedron et al., 2021) are decreased compared to control animals. Because these behaviors are possibly influenced by the motivation of animal, the decrease in them could represent the existence of distress, i.e., chronic pain. Alterations in behavioural performance have been also found in the measurement of depression-like (forced swimming test [FST], tail suspension test [TST], novelty-suppressed feeding test [NSFT], splash test, and sucrose consumption test), anxiety-like (elevated plus maze [EPM], elevated zero maze [EZM], and thigmotaxis), and cognitive impairment-like behaviors (passive avoidance test [PAT] and Morris water maze [MWM]) (da Silva Brum et al., 2021). It is possible that these behavioral alterations are, at least in part, caused by the existence of chronic pain, because pain has a negative impact on the emotion and cognitive function (Bushnell et al., 2013). The grimace scale, a methodology for rating facial expression (Langford et al., 2010; Sotocinal et al., 2011), was developed for measuring spontaneous ongoing pain in animals (Mogil et al., 2020). Recent studies have demonstrated that a substantial and long-lasting elevation of the grimace scale score occurs in RIM animal model (Nagakura et al., 2019; Tanei et al., 2020; Ferrarini et al., 2021). Furthermore, electroencephalography (EEG) recording has detected the

alterations in sleep EEG parameters in RIM animal model (Blasco-Serra et al., 2020), mimicking the sleep disturbance which is a prevalent comorbidity in patients with FM (Choy, 2015). The sleep alteration is possibly a consequence of the existence of chronic pain, because it is well known that chronic pain significantly interferes with sleep (Morin et al., 1998; Smith et al., 2000). Taken together, it is possible that the alterations of spontaneous behaviors and physical functions found in RIM animal model reflect the spontaneous ongoing pain in patients with FM.

Efficacy of Existing Drugs on RIM-induced Nociceptive Pain-Associated Phenotypes

The adequate relief of nociceptive pain in patients with FM has not been achieved by existing analgesic drugs, although various drugs are prescribed with reference to the guidelines (Chinn et al., 2016). Pregabalin (ligand at $\alpha_2\delta$ subunit of voltage-dependent calcium channels [VDCCs]), duloxetine (SNRI), and milnacipran (SNRI) have been mainstay options (Kia and Choy, 2017). Other drugs which stimulate monoamines-mediated signal, including tricyclic antidepressants and selective 5-HT reuptake inhibitors (SSRIs), have also been used. Those drugs have been assessed in RIM animal model by measuring evoked (reflex responses to external stimuli) and spontaneous pain

(spontaneous behaviors and physical functions) phenotypes. The assessment results and references cited for each result are summarized in Table 1. Many studies have shown that pregabalin and duloxetine attenuate evoked pain phenotypes (e.g., MA and heat HA). Drugs, which stimulate monoamines-mediated signals, such as lorcaserin (5-HT_{2C}-receptor [R] agonist), pramipexole (D_{2/3}-R agonist), ropinirole (D₂-R agonist), and vortioxetine (5-HT-R modulator) are also effective. Classical analgesics such as diclofenac (cyclooxygenase [cox] inhibitor) and buprenorphine (μ -R agonist) do not improve evoked pain phenotypes, although tramadol (μ -R agonist with SNRI activity) is effective for it.

The assessment using spontaneous pain phenotypes has also been conducted. The assessment results and references cited for each result are summarized in Table 1. Efficacy of pregabalin has been inconsistent, being effective or not on depression-like (FST, TST, NSFT, splash test, and sucrose consumption test), anxiety-like (EPM and thigmotaxis), and spontaneous (burrowing and exploratory locomotion) behaviors. Gabapentin (ligand at $\alpha_2\delta$ subunit as same as pregabalin) is effective in improving the alteration of facial expression (grimace scale). Duloxetine has been consistently effective in improving depression-like (FST and NSFT) and spontaneous (exploratory locomotion) behaviors, and facial expression (grimace scale). Fluoxetine (SSRI)

ameliorates the sleep alteration (EEG recording) which is possibly caused by the existence of chronic pain. Drugs, which stimulate monoamines-mediated signals, also improve spontaneous pain phenotypes. Pramipexole is effective in improving depression-like (FST, TST, and splash test), anxiety-like (EPM), and spontaneous (exploratory locomotion) behaviors. Ropinirole ameliorates depression-like (FST) and anxiety-like (EPM) behaviors. Vortioxetine improves anxiety-like (EPM) but not depression-like (FST) behavior. Either diclofenac or buprenorphine is not effective (grimace scale, FST, and sucrose consumption). Given the spontaneous pain phenotypes are more relevant to nociplastic pain in patients with FM, assessment of analgesic efficacy using them would be further implemented in RIM animal model.

Potential Therapeutic Approaches to Nociplastic Pain Based on Findings in RIM Animal Model

Pathophysiology Underlying RIM-Induced Nociplastic Pain-Associated Phenotypes

Pathophysiologies, i.e., biological processes occurring in the CNS, peripheral neurons, and skeletal muscles in RIM animal model and references cited for each

finding are summarized in Table 2. The chronic reduction in monoamines (NA, 5-HT, and DA), which simulates the hypothesis that the dysfunction of monoaminergic control is a trigger for the development of nociplastic pain, has sufficiently been verified mainly in the CNS, and in the skeletal muscle. Although the reduction of monoamines itself might be directly responsible for the RIM-induced nociplastic pain-associated phenotypes, it is probable that biological processes occurring after this trigger are implicated as crucial factors.

In the CNS, the upregulation of pronociceptive neurotransmitters and their receptors, transporters, and neuronal activity markers has been detected. The increase of N-methyl-D-aspartate (NMDA)-R has been verified in the brain and spinal cord. The increase in GluN2B-containing NMDA-R is accompanied by the increase of phosphorylated α -3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-R, neuronal activity markers (postsynaptic density protein 95 [PSD-95] and Ca^{2+} /calmodulin-dependent protein kinase II [CaMKII]), nitrosative stress marker (inducible nitric oxide synthase [iNOS]), and apoptosis mediator (caspase-3). The increase of an excitatory transmitter, glutamate is also demonstrated in the brain and spinal cord. These findings suggest that the glutamate-mediated excitatory synaptic transmission and subsequent biological processes including nitrosative stress are enhanced. A study has found the

increase of extrasynaptically localized $\alpha 5$ γ -aminobutyric acid (GABA)_A-R and the decrease of neuronal K⁺-Cl⁻ co-transporter 2 (KCC2) which maintains a low intracellular Cl⁻ concentration in the spinal cord. Given the function of GABA_A-R switches from inhibition to excitation of neurons due to the decrease of KCC2 (Delgado-Lezama et al., 2021), the increase of $\alpha 5$ GABA_A-R with decreased KCC2 could cause the activation of neurons responsible for pain signal transmission in the spinal cord. According to a study investigating the nociceptin/orphanin FQ (N/OFQ) system, the precursor of N/OFQ is increased in the spinal cord, while nociceptin opioid receptor (NOP-R) is increased in the thalamus and hypothalamus. There is sufficient evidence that the level of oxidative/nitrosative stresses is elevated. The significant increase of oxidative stress markers (e.g., lipid peroxidation product [LPO], malondialdehyde [MDA], thiobarbituric acid reactive substance [TBARS], hydrogen peroxide [H₂O₂]) and nitrosative stress markers (e.g., inducible nitric-oxide synthase [iNOS], nitrotyrosine, and nitric oxide [NO]), and the decrease of endogenous antioxidant enzymes (e.g., superoxide dismutase [SOD], glutathione [GSH], and catalase [CAT]) have been detected in the cortex, hippocampus, thalamus, and spinal cord. The elevated oxidative/nitrosative stresses could be associated with the inflammatory process in the CNS by the oxidative/nitrosative stress-neuroimmune

interaction and responsible for the development of pain phenotypes (Grace et al., 2021). Evidence for the presence of inflammatory process in the CNS has been accumulating. The increased expression of bradykinin-related peptide (proinflammatory mediator) in the cortex and kinin B1 and B2 receptors in the cortex and spinal cord has been demonstrated. Another study demonstrates that substance P increases in the cortex and hippocampus. A study investigating the adenosine triphosphate (ATP) system has shown that P2X7-R (ATP-gated ion channel), nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome, and inflammatory cytokines (IL-1 β and IL-18) are increased in the brain and spinal cord. The activation of astrocyte and microglial cell is also detected in the hypothalamus and spinal cord. It is possible that the ATP-induced overactivation of P2X7-R on glial cells exacerbates pain by activating the NLRP3 inflammasome pathway and subsequently causing the release of IL-1 β and pro-IL-18. Other studies have shown the increase of pro-inflammatory cytokines (tumor necrosis factor- α [TNF- α] and IL-1 β) and nuclear factor- κ B (NF- κ B) (proinflammatory transcription factor) in the brain regions. The activation of immune cells (microglia, astrocyte, and mast cell) has been found in the brain and spinal cord. Such glial cell activation could induce the sensitization of neurons by the release of proinflammatory cytokines (IL-1 β and TNF- α) and growth factors (nerve growth factor

[NGF] and vascular endothelial growth factor [VEGF]). Indeed, a recent study has detected the sensitization in superficial dorsal horn (SDH) neurons by *in vivo* electrophysiological recording (Taguchi et al., 2015). The increase of mediators responsible for programmed cell death such as p65 subunit of NF κ B, caspase-1, caspase-3, apoptosis-associated speck-like protein, poly-ADP-ribose polymerase (PARP), and the decrease of anti-apoptotic factor (Bcl-2) have been found in the brain and spinal cord. It is possible that this elevation of apoptotic signals is associated with the above-mentioned increase of oxidative/nitrosative stress (Kannan and Jain, 2000).

In peripheral neurons, alterations of expression in pronociceptive neurotransmitters and their receptors, and channels have been demonstrated. The increase of $\alpha 5$ GABA_A-R, which might lead to the enhancement of pain signal transmission as suggested in the CNS, has been found also in the dorsal root ganglion (DRG). A study has found the increase of expression of acid-sensing ion channel-3 (ASIC3) which has a crucial role in the pain caused by tissue acidosis (Wu et al., 2012). It is possible that ASIC3 overexpression of ASIC3 is associated with the sensitization of afferent neurons. The expression of NOP-R is increased in the DRG as well as in the CNS. B1 and B2 receptors, and bradykinin-related peptide are increased in the sciatic nerve, suggesting the presence of neuroinflammatory process. The activation of mast cell alongside with

the increase of growth factors (NGF and VEGF) in the sciatic nerve could be responsible for the development of peripheral sensitization based on a key role of mast cells in the inflammatory process and modulation of neural activity (Traina, 2019).

In the skeletal muscle, the reduction of monoamines (NA, 5-HT, and DA) has been detected in the gastrocnemius muscle and soleus muscle. As in the CNS, NOP-R and precursor of N/OFQ are increased in the masseter muscle. The elevation of oxidative stress has been verified by several studies. The increase of oxidative stress markers and the decrease of antioxidant enzymes have been detected in the gastrocnemius muscle and extensor digitorum longus muscle. The decrease of peroxisome proliferator-activated receptor-gamma coactivator-1 α (PGC-1 α ; regulator of mitochondrial function), mitofusin2 (Mfn2; membrane protein of mitochondria), and coenzyme Q10 (CoQ10; key component of the mitochondrial respiratory chain) suggests that the elevation of oxidative stress is possibly due to mitochondrial dysfunction. The elevation of NLRP3 inflammasome found in the gastrocnemius muscle could suggest the presence of inflammatory process. In addition to the biochemical alterations mentioned above, modified post-occlusive reactive hyperemia, a physiological function, has also been detected in the anterior tibialis muscle. It is possible that such alteration in local regulation of blood flow might be associated with the development of muscle pain.

Possible Therapeutic Approaches to Nociceptive Pain

The efficacy of potential therapeutic approaches on evoked and spontaneous pain phenotypes in RIM animal model, and references cited for each finding are summarized in Table 3. The first category of approach is the functional modification of specific molecules which expression is distinctly altered following the reserpine treatment as summarized in Table 2. Both of des-Arg⁹-[Leu⁸]-bradykinin (DALBk; B1-R antagonist) and icatibant (B2-R antagonist) attenuate evoked pain phenotype (MA and cold HS), although only DALBk attenuates spontaneous pain phenotype (burrowing). This is in line with the evidence that bradykinin is involved in the pathogenesis of chronic musculoskeletal pain (Pinheiro et al., 2013) and peripheral sensitization (Petho and Reeh, 2012). UFP-101 and SB-612111 (antagonists for NOP-R) reverse both of evoked (MA and heat HA) and spontaneous pain (FST) phenotypes, suggesting that the N/OFQ-NOP-R system plays an important role in the pathophysiology at the level of the CNS, peripheral neuron, and skeletal muscle. Brilliant blue G (BBG; antagonist for P2X7-R) prevents the activation of immune cells (microglia and mast cell), inhibits the expression of NLRP3 and release of proinflammatory mediators (IL-1 β and IL-18) in the CNS, and attenuates both of evoked (MA and heat HA) and spontaneous pain (FST

and TST) phenotypes. These findings suggest that the inhibition of ATP-induced overactivation of P2X7-R on immune cells with the antagonist is an effective approach to suppress the neuroinflammation in the CNS and consequently attenuate chronic pain. Intrathecal injection of L-655708 and TB 21007 ($\alpha 5$ GABA_A-R inverse agonists) ameliorates evoked pain phenotype (MA and mechanical HA). Given the decreased expression of KCC2 causes a functional switch of GABA_A-R from inhibition to excitation of neurons (Fukuda, 2020), the inhibition of spinal $\alpha 5$ GABA_A-R with inverse agonists could inhibit the spinal neurons responsible for pain signal transmission. APETx2 (blocker of ASIC-3) recovers the overexpression of ASIC-3 mRNA in the DRG, inhibits mechanical responses of mechanoresponsive C-fibers, and attenuates MA. This is consistent with that ASIC-3 is abundantly expressed in sensory neurons and mediates the acidosis-induced sustained current (Dulai et al., 2021). Minocycline, which is commonly used to inhibit microglial activity (Kobayashi et al., 2013), reduces the sensitivity of SDH neurons in the spinal cord and attenuates MA. Because the activation of microglia leads to the release of proinflammatory mediators (Ji et al., 2018) which play a crucial role in promoting widespread chronic pain via central sensitization, the inhibition of microglial activity could be an effective approach to prevent the chronic pain development.

The second category of approach is to target the molecules, which have been considered responsible for other major categories of chronic pain (i.e., chronic inflammatory pain and neuropathic pain). Phenylzoline, 2-(2-benzofuranyl)-2-imidazoline (2-BFI), and CR4056 (imidazoline I2-R agonists) attenuate both of evoked (MA) and spontaneous pain (FST) phenotypes. It has been demonstrated that I2-R agonists including 2-BFI and CR405 attenuate MA in animal models of neuropathic pain and chronic inflammatory pain through the modulation of serotonergic and noradrenergic neuronal activities (Siemian et al., 2018), although the role of I2-R in the pain signal transmission in RIM animal model remains to be elucidated. SB-366791 and α -spinasterol (transient receptor potential vanilloid type 1 [TRPV1] antagonists) recover both of evoked (MA and heat HA) and spontaneous pain (FST) phenotypes. Also, the desensitization of TRPV1-positive fibers by subcutaneous injection of resiniferatoxin attenuates evoked pain phenotype (MA and heat HA). This finding is consistent with the evidence that TRPV1 is responsible for the detection of noxious stimuli and development of chronic pain state (Szallasi et al., 2007; Abdelhamid et al., 2014), and that TRPV1 is validated as a therapeutic target for neuropathic and chronic inflammatory pain (Iftinca et al., 2021). Ph α 1 β (spider toxin) significantly attenuates evoked pain phenotype (mechanical HA and heat HA) but not spontaneous pain

phenotype (FST and sucrose consumption). This is in line with a previous report that intrathecally administered Ph α 1 β inhibits mechanical HA in an animal model of neuropathic pain possibly by inhibiting N-type voltage-dependent calcium channel (VDCC) and suppressing the release of pronociceptive neurotransmitters in the spinal cord (Rigo et al., 2013). Tx3-3, another spider toxin, improves both evoked (MA and heat HA) and spontaneous pain (locomotor activity [LA] and FST) phenotypes. This coincides with a literature showing that intrathecal injection of Tx3-3 exhibits antinociception in animal models of neuropathic pain (Dalmolin et al., 2011) possibly by the blockade of P/Q and R type VDCCs and inhibition of the release of glutamate in the spinal cord (Gomez et al., 2002). Astellas Pharma Inc has demonstrated that a series of compounds, which are effective in animal models of neuropathic and/or chronic inflammatory pain, also reduce the nociplastic pain-associated phenotypes in RIM animal model. ASP8477 (inhibitor of fatty acid amide hydrolase) reduces evoked pain phenotype (muscle HA), being consistent with a previous study which demonstrates that ASP8477 elevates the anandamide (endogenous cannabinoid) concentration in both plasma and brain and attenuates evoked pain phenotype in animal models of neuropathic and chronic inflammatory pain (Watabiki et al., 2017). ASP8062 (positive allosteric modulator [PAM] at GABA_B receptor) reduces evoked (muscle HA) and

spontaneous pain (sleep disturbance) phenotypes. Of note, it recovers the altered sleep EEG parameters, being consistent with that gamma-hydroxybutyrate, a compound having GABA_B-R agonist activity, relieves pain symptoms and sleep disturbance in patients with FM (Russell et al., 2011). ASP3662 (inhibitor of 11 β -hydroxysteroid dehydrogenase [HSD]) reduces muscle HA, being consistent with its analgesic effect in animal models of neuropathic pain (Kiso et al., 2018b) and the evidence that intrathecal injection of glucocorticoid-R antagonist attenuates MA and heat HA in an animal model of neuropathic pain (Wang et al., 2004). Given that 11 β -HSD1 is an enzyme responsible for the intracellular regeneration of glucocorticoids also in the CNS (Moisan et al., 1990), the reduction of active glucocorticoid in the CNS could be a mechanism for analgesic action of ASP3662. Moreover, ASP0819 (opener of a calcium-activated potassium channel [KCa3.1 channel]) reduces both spontaneous activity and mechanically evoked responses in sensory neurons in the electrophysiological recording, and attenuates evoked pain phenotype (muscle HA). This is consistent with a previous study using a chemically-induced animal pain model, which demonstrates that KCa3.1 channel has an inhibitory role in the pain signal transmission (Lu et al., 2017).

The third category of approach is the supplementation of nutrition to correct the disrupted nutritional balance. An important pathophysiology found in patients with FM

is that deficiency of essential nutrients (e.g., amino acids, vitamins, magnesium, and selenium) leads to dysfunction of pain inhibitory mechanisms and exacerbates symptoms such as muscular pain (Bjørklund et al., 2018). Supplementation of selenium (essential trace element with antioxidant activity) as organic selenium compounds, such as p, p'-methoxyl-diphenyl diselenide, α - (phenylselanyl) acetophenone (PSAP), and m-trifluoromethyl-diphenyl diselenide ($[m\text{-CF}_3\text{-PhSe}]_2$), reduce the oxidative stress and attenuate both of evoked (MA and heat HA) and spontaneous pain (FST) phenotypes. This result could be associated with a study which demonstrates that patients with FM take less minerals including selenium than control (Batista et al., 2016). Because the optimal nutrition reduces the pain intensity in patients with FM (Bjørklund et al., 2018), supplementation of deficient essential nutrients could be a reasonable approach to relieve nociplastic pain.

The fourth category of approach is the improvement of physical constitution by natural substances. Composite factors including oxidative/nitrosative stress and inflammatory process are underlying the nociplastic pain-associated phenotypes in RIM animal model. Although an orthodox way for controlling such composite pathophysiological factors would be to identify a specific hub molecule in the molecular cascade or network, it might be challenging to identify such molecule to achieve

sufficient pain relief. Thus, an alternative approach could be the intake of natural substances such as plant natural products and endogenously occurring substances (e.g., hormone).

Plant natural products possessing biological activities have shown beneficial effects on the nociplastic pain-associated phenotypes as well as the underlying pathophysiological factors in RIM animal model. Coumarin derivatives are phenolic substances enriched in various plants. Some of them have beneficial properties including antioxidant and anti-inflammatory activities (Garg et al., 2020). Curcumin from *Curcuma longa* rectifies the altered biological processes (monoamines concentration, oxidative stress, inflammatory process, and pronociceptive neurotransmitter) in the CNS, and attenuates both of evoked (MA and mechanical HA) and spontaneous pain (FST) phenotypes. Imperatorin from *Angelica dahurica* rectifies the overexpression of NMDA receptor and NF κ B in the CNS, and ameliorates both of evoked (MA and mechanical HA) and spontaneous pain (FST, EPM, PAT, MWM, and LA) phenotypes, possibly by inhibiting the NMDA-R/Ca²⁺-mediated extracellular signal-regulated kinase pathway. Esculetin from various plants including *Aesculus hippocastanum*, Skimmetin from *Hydrangea paniculata*, and Daphnetin from *Thymelaeaceae* family plants correct the level of monoamines, TBARS, GSH, and

MAO-A in the brain, and attenuate both of evoked (MA and mechanical HA) and spontaneous pain (FST and MWM) phenotypes. Other kind of substances from plant sources have also been assessed. Gentiopicroside, a secoiridoid from *Gentiana lutea* possessing antioxidant and anti-inflammatory properties, reverses the altered level of monoamines and GluN2B-containing NMDA receptors in the amygdala and recovers both of evoked (MA and heat HA) and spontaneous pain (FST and OFT) phenotypes. β -carotene (carotenoid essential in plant photosynthesis) has been taken as dietary antioxidant. Its administration reduces oxidative/nitrosative stresses, increases monoamines in the cortex, and attenuates evoked pain phenotype (mechanical HA). Fisetin and resveratrol (polyphenols possessing antioxidant activity) has been taken from plant foods including fruits, vegetables, herbs, and wine. Their administration reduces the oxidative stress in the CNS, and attenuates both of evoked (mechanical HA) and spontaneous pain (FST) phenotypes. Aegeline (alkaloid occurring in *Aegle marmelos*) has an antioxidant activity. Its administration reduces oxidative/nitrosative stresses and suppresses both of evoked (MA and mechanical HA) and spontaneous pain (FST) phenotypes. *Angelica archangelica* (aromatic herb) possesses an antioxidant activity (Prakash et al., 2015) and has been used as an alternative medicine. Administration of the extract from its root lowers oxidative/nitrosative stresses in the

brain and spinal cord and attenuates both of evoked (mechanical HA) and spontaneous pain (PAT, MWM, and LA) phenotypes.

Functional ingredients in plant foods have been shown to reduce pain-associated phenotypes in RIM animal model. Ferulic acid, a phytochemical commonly found in fruit and vegetables with antioxidant properties, reverses the alteration in oxidative/nitrosative stresses, inflammatory process, and apoptosis, recovers the decrease of monoamines in the hippocampus and frontal cortex, and attenuates both of evoked (MA and heat HA) and spontaneous pain (FST and TST) phenotypes. Echinocystic acid, a triterpene enriched in various herbs displaying antioxidant and anti-inflammatory activities, suppresses the glutamate-mediated excitatory synaptic transmission and subsequent biological processes in the hippocampus, and attenuates both of evoked (mechanical HA) and spontaneous pain (FST, TST, and OFT) phenotypes. The above-mentioned findings suggest that the use of plant natural products possessing biological activities especially, antioxidant and anti-inflammatory effects, would be an effective approach to reduce the nociplastic pain in patients with FM.

The supplementation of naturally occurring substances in the human body has also been tested in RIM animal model. Melatonin is a hormone, which is secreted by the

pineal body, has radical scavenging, anti-inflammatory, and antioxidant activities. While RIM animal model exhibits the increase of oxidative stress and inflammatory mediators, and the decrease of substances responsible for mitochondrial homeostasis in the gastrocnemius muscle, oral administration of melatonin rectifies the alteration of all the parameters and improves spontaneous pain phenotype (voluntary motor activity). Administration of melatonin in combination with folic acid, a vitamin with antioxidant properties (Stanhewicz and Kenney, 2017), rectifies the altered parameters associated with oxidative/nitrosative stresses, reduces activity of immune cells in the brain, and attenuates both of evoked (MA and heat HA) and spontaneous pain (FST) phenotypes. CoQ10 (endogenous antioxidant) is an essential component in the mitochondrial electron transport chain (Hernández-Camacho et al., 2018). Its administration prevents the mitochondrial dysfunction and overproduction of reactive oxygen species (ROS) in the spinal cord and skeletal muscle, and attenuates both of evoked (MA and cold HS) and spontaneous pain (FST and thigmotaxis) phenotypes. Aspirin-triggered resolvin D1 (AT-RvD1) and AT-RvD2 are analogs of the naturally occurring D series Rvs, which possess potent anti-inflammatory actions (Shan et al., 2020). Their administration significantly increases monoamines (5-HT and DA), decreases glutamate in the CNS, and reduces both of evoked (MA and heat HA) and spontaneous pain (FST) phenotypes.

The beneficial effects of the above-mentioned natural substances could be due to modulation of the physical constitution, mainly by reducing the elevated oxidative stress, rather than the effect on a specific molecular target. Although an acute or sharp effect might not be strongly expected for such natural substances, they could potentially work as alternative therapies for the attenuation of nociplastic pain.

The fifth category of approach is nonpharmacological interventions. The treatment of FM begins with nonpharmacological interventions including education, exercise, psychotherapy and acupuncture, (Macfarlane et al., 2017), although their efficacy varies depending on individual patient's predominant symptom (Kundakci et al., 2021). Recently, nonpharmacological therapeutic interventions have been assessed in RIM animal model. Electroacupuncture at 100 Hz with needles inserted into the acupoints recovers the decrease of 5-HT in the dorsal raphe nucleus and attenuates both of evoked (MA) and spontaneous pain (EZM and OFT) phenotypes. Exercise (free gait in shallow water 5 times per week) facilitates the recovery of decreased LA (spontaneous pain phenotype). Another protocol of exercise (four-week aerobic and strength) reduces the number of unmyelinated fibers in the DRG and attenuates both of evoked (MA and cold HS) and spontaneous pain (spontaneous pain score) phenotypes. These results demonstrate that RIM animal model is useful in assessing efficacy of

nonpharmacological interventions. Further investigations of nonpharmacological therapies in RIM animal model would contribute to the verification and optimization of their efficacy, and the clarification of their mechanism.

Translation of the Efficacy from the RIM Animal Model to Patients

Potential therapeutic approaches to nociplastic pain have been identified as described above, although the translation of their efficacies from RIM animal model to patients with nociplastic pain remains to be addressed. There are critical points to be considered in the process of translation. First, many therapeutic approaches correct the reserpine-induced alteration in the expression of the molecule involved in the biological process underlying the nociplastic pain-associated phenotype. It is restrictive that most of such molecular alterations are detected in the difficult-to-measure regions (i.e., brain, spinal cord, and skeletal muscle) when assuming measurement in patients. It would be valuable to investigate whether such molecular alterations also happen in collectible body fluids such as blood and urine in RIM animal model. If that is the case, such molecules may be used as pharmacodynamic biomarkers to monitor the efficacy of therapeutic approaches. The availability of such biomarkers would promote the

prospective translation from animal to patients in the processes of clinical studies, e.g., dose-setting and interpretation of results.

Second, potential therapeutic approaches have mostly been evaluated using the evoked pain phenotypes in RIM animal model, although spontaneous ongoing pain is the main complaint in patients with nociplastic pain. Because this gap regarding the endpoint measure can cause failure in the translation of analgesic efficacy, the therapeutic approach that attenuates spontaneous ongoing pain-associated phenotypes should be pursued and given priority for better translation. As described above, the decrease in naturally occurring behaviors, alterations in depression-like, anxiety-like, and cognitive impairment-like behaviors, sleep disturbance, and alteration in facial expression have been detected in RIM animal model. It is possible that these endpoint measures, at least in part, reflect the intensity of spontaneous ongoing pain, although further validation studies would be warranted. The implementation of well-validated measures reflecting spontaneous ongoing pain in patients with FM into the RIM model would contribute to the better translation of efficacies from animals to patients.

Conclusions

Biological processes including the chronic reduction of monoamines, increase of oxidative/nitrosative stresses and inflammatory mediators, upregulation of pronociceptive neurotransmitters and their receptors, increase of trophic factors, enhancement of the apoptotic pathway, sensory nerve sensitization, and activation of immune cells in the central and/or peripheral regions, underly the nociplastic pain-associated phenotypes in RIM animal model. Studies using RIM animal model have identified potential therapeutic approaches to nociplastic pain, i.e., 1) functional modification of specific molecules which expression is distinctly altered following the monoamine reduction, 2) targeting the molecules which are responsible for other major categories of chronic pain (i.e., chronic inflammatory pain and neuropathic pain), 3) supplementation of nutrition to correct the disrupted nutritional balance, 4) improvement of physical constitution by natural substances, and 5) nonpharmacological interventions. Translation of the analgesic efficacy of such approaches from RIM animal model to patients remains a challenge. Successful translation would lead to better therapies for patients with nociplastic pain.

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TABLE 1. Efficacy of Existing Drugs on RIM-Induced Nociceptive Pain-Associated Phenotypes

Drugs (mechanism)	Species	Efficacy (evoked pain phenotypes)	Efficacy (spontaneous pain phenotypes)	Literature
Pregabalin ($\alpha_2\delta$ subunit ligand)	Rat	MA (+), Muscle HA (+)	-	(Nagakura et al., 2009; Kiso et al., 2018a)
		MA (+), Mechanical HA (+)	FST (+), NSFT (-)	(Gonzalez-Soler et al., 2020)
		MA (+), Heat HA (+)	FST (-)	(de Souza et al., 2014; Klein et al., 2014)
	Mouse	MA (+), Heat HA (+)	FST (-), LA (-)	(Peres Klein et al., 2016)
		MA (+), Cold HS (+)	Burrowing (-), Thigmotaxis (-)	(Brusco et al., 2019)
		MA (+)	FST (+), LA (+)	(Pedron et al., 2021)
		Mechanical HA (+)	FST (-), Sucrose consumption (-)	(Garcia Mendes et al., 2021)
		MA (+), Heat HA (-)	FST (+), EPM (+), Splash test (+)	(Martins et al., 2022)
Gabapentin $\alpha_2\delta$ subunit ligand)	Rat	MA (+)	Grimace scale (+)	(Nagakura et al., 2019)
Duloxetine (SNRI)	Rat	MA (+), Muscle HA (+)	-	(Nagakura et al., 2009)
		Muscle HA (+)	-	(Murai et al., 2015; Kiso et al., 2018a)
		MA (+)	FST (+), NSFT (+)	(Zhang et al., 2016)
	Mouse	MA (+)	Grimace scale (+)	(Nagakura et al., 2019)
		MA (+), Mechanical HA (+)	FST (+), NSFT (+)	(Gonzalez-Soler et al., 2020)
Ammoxetine	Rat	MA (+)	-	(Zhang et al., 2016)

(SNRI)				
Pramipexole (D ₂ /D ₃ -R agonist)	Rat	MA (+), Muscle HA (+)	-	(Nagakura et al., 2009)
		MA (+)	FST (+), LA (+)	(Pedron et al., 2021)
	Mouse	MA (+), Heat HA (+)	TST (+), Splash test (+), EPM (+)	(Martins et al., 2022)
Ropinirole (D ₂ -R agonist)	Mouse	MA (+), Heat HA (-)	FST (+)	(Sałat and Furgała-Wojas, 2021)
Vortioxetine (5-HT-R modulator)	Mouse	MA (+), Heat HA (-)	FST (-), FPT (+)	(Sałat and Furgała-Wojas, 2021)
Fluoxetine (SSRI)	Rat	-	Sleep alteration (+)	(Hernandez-Leon et al., 2019)
Lorcaserin (5-HT _{2C} -R agonist)	Rat	Muscle HA (+)	-	(Ogino et al., 2013)
Diclofenac (Cox inhibitor)	Rat	MA (-), Muscle HA (-)	-	(Nagakura et al., 2009)
		MA (-)	Grimace scale (-)	(Nagakura et al., 2019)
	Mouse	-	FST (-), Sucrose consumption (-)	(Garcia Mendes et al., 2021)
Buprenorphine (μ-R agonist)	Rat	MA (-)	Grimace scale (-)	(Nagakura et al., 2019)
Tramadol (μ-R agonist/SNRI)	Rat	MA (+)	-	(Kaneko et al., 2014)
Mexiletine (Nav inhibition)	Rat	MA (-), Muscle HA (-), Cold HA (-)	-	(Nagakura et al., 2012)

(+): significantly effective compared to vehicle, (-): not significantly effective compared to vehicle, -; not assessed

TABLE 2

Pathophysiology Underlying RIM-Induced Nociceptive Pain-Associated Phenotypes

Pathophysiology (Site)	Species	Biochemical alteration <Primary region>	Literature
Reduction of monoamines (CNS)	Rat	[↓] NA, 5-HT, DA <cortex, hippocampus, thalamus, spinal cord>	(Nagakura et al., 2009; Oe et al., 2010; Arora et al., 2011; Arora and Chopra, 2013; Kiso et al., 2018a; Yao et al., 2020; Elkholy et al., 2021)
	Mouse		(Xu et al., 2013; de Souza et al., 2014; Klein et al., 2014; Liu et al., 2014; Li et al., 2016; Fischer et al., 2020; Singh et al., 2020; Singh et al., 2021c)
Receptors, channel, and transmitters (CNS)	Rat	[↑] $\alpha 5$ - GABA _A -R <spinal cord> [↓] KCC2 <spinal cord>	(De la Luz-Cuellar et al., 2019)
	Mouse	[↑] N/OFQ precursor <spinal cord> [↑] NOP-R <thalamus, hypothalamus> [↓] NOP-R <cortex>	(Dagnino et al., 2019)
		[↑] NMDA-R, NF κ B p65, NF κ B-R <brain, spinal cord>	(Kaur et al., 2019)
		[↑] GluN2B-containing NMDA-R, caspase-3 < amygdala>	(Liu et al., 2014)
		[↑] GluN2B-containing NMDA-R, p- GluA1-Ser831, PSD-95, CaMKII, 5- HT _{2A} -R <hippocampus> [↓] 5-HT _{1A} -R <hippocampus>	(Li et al., 2016)
		[↑] Glutamate <thalamus, spinal cord>	(Klein et al., 2014)
		[↑] Glutamate <brain>	(Singh et al., 2020; Singh et al.,

			2021b; Singh et al., 2021c)
Oxidative/nitrosative stresses (CNS)	Rat	[↑] LPO, Nonprotein thiols, Nitrotyrosine <brain> [↓] SOD, CAT <brain>	(Fusco et al., 2019)
		[↑] MDA, H ₂ O ₂ , NO <cortex> [↓] GSH <cortex>	(Elkholy et al., 2021)
		[↑] MDA <cortex>	(Oliveira et al., 2016)
	Mouse	[↑] TBARS, MAO-A, iNOS <Brain> iNOS <spinal cord> [↓] GSH <brain>	(Singh et al., 2020; Singh et al., 2021a; Singh et al., 2021b; Singh et al., 2021c)
		[↑] LPO, Nitrite <cortex, hippocampus> [↓] GSH, SOD <cortex, hippocampus>	(Xu et al., 2013)
		[↑] MDA, NO <thalamus> [↑] ROS <spinal cord> [↓] GSH, SOD <thalamus>	(Yao et al., 2020)
		[↑] iNOS <hippocampus>	(Li et al., 2016)
		[↑] ROS <CSF>	(Peres Klein et al., 2016)
		[↑] ROS <spinal cord> [↓] O ₂ flux <spinal cord>	(Brum et al., 2020)
		[↑] MDA, Nitrite <cortex, hippocampus> [Act] MAO <cortex, hippocampus>	(Sousa et al., 2018)
		[↑] TBARS, 4-Hydroxynonenal modified protein <hippocampus>	(Schossler Garcia et al., 2021b)
Proinflammatory factors (CNS)	Rat	[↑] P2X7R, NLRP3, IL-1β, IL-18 <brain, spinal cord>	(D'Amico et al., 2021)
		[↑] IL-1β, TNF-α, NFκB <cortex, hippocampus>	(Arora et al., 2011; Arora and Chopra, 2013)
		[↑] IL-1β, TNF-α <brain>	(Fusco et al., 2019)
		[↑] Substance P <cortex, hippocampus>	(Arora et al., 2011; Arora and Chopra, 2013)
	Mouse	[↑] B1R, B2R, Bradykinin-related peptide <brain, spinal cord>	(Brusco et al., 2019)
		[↑] IL-1β, TNF-α, NFκB <cortex,	(Xu et al., 2013)

		hippocampus>	
		[↑] IL-1 β , TNF- α <brain>	(Singh et al., 2020; Singh et al., 2021b; Singh et al., 2021c)
Immune cells (CNS)	Rat	[Act] Microglia, Mast cell <brain> [↑] NGF, VEGF <brain>	(Fusco et al., 2019)
		[Act] Microglia <spinal cord>	(Taguchi et al., 2015) (Uta et al., 2021)
		[Act] Astrocyte, Microglia, Mast cell <spinal cord>	(D'Amico et al., 2021) (De la Luz-Cuellar et al., 2019)
		[Act] Schwann cell <spinal cord>	(Yao et al., 2020)
	Mouse		
Apoptosis (CNS)	Rat	[↑] Caspase-3, NF- κ B <cortex, hippocampus>	(Arora et al., 2011; Arora and Chopra, 2013)
		[↑] PARP <brain>	(Fusco et al., 2019)
		[↑] Apoptosis-associated speck-like protein, Caspase-1 <spinal cord>	(D'Amico et al., 2021)
	Mouse	[↑] PARP <hippocampus>	(Schossler Garcia et al., 2021b)
		[↑] Caspase-3, NF- κ B p65 <cortex, hippocampus>	(Xu et al., 2013)
		[↑] Caspase-3< hippocampus >	(Li et al., 2016)
		[↑] Caspase-3, GluN2B-containing NMDA-R <amygdala>	(Liu et al., 2014)
		[↑] NF κ B p65 <spinal cord>	(Kaur et al., 2019)
Receptors, channel, and transmitters (peripheral neuron)	Rat	[↑] α 5-GABA _A -R <DRG>	(De la Luz-Cuellar et al., 2019)
		[↑] ASIC-3 <DRG>	(Taguchi et al., 2015)
		[↑] NOP-R <DRG>	(Dagnino et al., 2019)
Proinflammatory factors (peripheral neuron)	Mouse	[↑] B1R, B2R, Bradykinin-related peptide <sciatic nerve>	(Brusco et al., 2019)
Immune cells (peripheral neuron)	Rat	[Act]Mast cell [↑] NGF, VEGF <sciatic nerve>	(Fusco et al., 2019)
Monoamines	Rat	[↓] NA, 5-HT, DA <gastrocnemius	(Nagakura et al., 2018)

(skeletal muscle)		muscle>	
	Mouse	[↓] NA, DA <soleus muscle>	(Brum et al., 2020)
Receptors, transporters, and transmitters (skeletal muscle)	Mouse	[↑] NOP-R, Prepronociceptin <masseter muscle>	(Dagnino et al., 2019)
Oxidative stress (skeletal muscle)	Rat	[↑] Total thiol, NLRP3 <gastrocnemius muscle> [↓] SOD, CAT, PGC-1 α , Mfn2, CoQ10 <gastrocnemius muscle>	(Favero et al., 2017; Favero et al., 2019)
	Rat	[↑] MDA <extensor digitorum longus muscle> [↓] SOD, CAT <extensor digitorum longus muscle>	(Hassan et al., 2021)
	Mouse	[↑] ROS, TBARS <gastrocnemius muscle> [↓] O ₂ flux, SOD, CAT, PGC-1 α , Mfn2, CoQ10 <soleus muscle>	(Brum et al., 2020)
Local regulation of blood flow (skeletal muscle)	Rat	Alteration in post-occlusive reactive hyperemia <anterior tibialis muscle>	(Souza-Silva et al., 2021)

[↑]: Increase, [↓]: Decrease, [Act]: Activation

TABLE 3

Potential Therapeutic Approaches to Nociceptive Pain Based on Studies in RIM Animal Model

Substances	Mechanism	Species	Effects on pathophysiology	Evoked pain phenotypes	Spontaneous Pain phenotypes	Literature
DALBk	B1-R blockade	Mouse	(↓) Signal transmission mediated by B1-R	MA (+), Cold HS (+)	Burrowing (+), Thigmotaxis (-)	(Brusco et al., 2019)
Icatibant	B2-R blockade	Mouse	(↓) Signal transmission mediated by B2-R	MA (+), Cold HS (+)	Burrowing (-) Thigmotaxis (-)	(Brusco et al., 2019)
UFP-101, SB-612111	NOP-R blockade	Mouse	Improvement of N/OFQ-NOP-R tonus <CNS, peripheral neurons, skeletal muscle>	MA (+), Heat HA (+)	FST (+)	(Dagnino et al., 2019)
BBG	P2X7R blockade	Rat	(↓) Activity of immune cells <spinal cord> (↓) Proinflammatory mediators, NLRP3 <spinal cord>	MA (+), Heat HA (+)	FST (+), TST (+)	(D'Amico et al., 2021)
L-655708, TB 21007	$\alpha 5$ GABA _A -R inverse agonist	Rat	(↓) $\alpha 5$ GABA _A -R activity <spinal cord>	MA (+), Mech. HA (+)	-	(De la Luz-Cuellar et al., 2019)
APETx2	ASIC3 inhibition.	Rat	(↓) Response of mechanoresponsive sensory neurons	MA (+)	-	(Taguchi et al., 2015)

Minocycline	Microglia inhibition	Rat	(↓) Sensitivity of SDH neurons <spinal cord>	MA (+)	-	(Taguchi et al., 2015)
CR4056	I ₂ -R agonist	Rat	Modulation of I ₂ -R-mediated pain signal <brain>	MA (+)	FST (+)	(Siemian et al., 2019)
SB-366791, α -spinasterol	TRPV1 blockade	Mouse	(↓) TRPV1-mediated signal transmission	MA (+), Heat HA (+)	FST (+)	(Fischer et al., 2020)
Resiniferatoxin	TRPV1 irreversible binding	Mouse	Desensitization of sensory neurons	MA (+), Heat HA (+)	-	(Fischer et al., 2020)
Pha1 β toxin	N-type VDCC inhibition	Mouse	(↓) Neurotransmitter release <brain, spinal cord>	Mech. HA (+), Heat HA (+)	FST (-), Sucrose consumption, (-)	(de Souza et al., 2014) (Garcia Mendes et al., 2021)
Tx3-3 toxin	P/Q- and R-type VDCC inhibition	Mouse	(↑) 5-HT, DA <brain>	MA (+), Heat HA (+)	LA (+), FST (+)	(Pedron et al., 2021)
ASP8477	FAAH inhibition	Rat	(↓) Anandamide catabolism	Muscle HA (+)	-	(Kiso et al., 2020)
ASP8062	GABA _B -R PAM	Rat	(↑) GABA _B -R function <CNS>	Muscle HA (+)	-	(Murai et al., 2019)
ASP3662	Inhibition of 11 β -HSD1	Rat	(↓) Glucocorticoids <CNS>	Muscle HA (+)	-	(Kiso et al., 2018b)
ASP0819	KCa3.1 channel activation	Rat	(↓) Activity of sensory A δ -fibers	Muscle HA (+)	-	(Takeshita et al., 2021)
p, p'-	Selenium	Rat	(↑) NF-E2-related factor 2	MA (+), Heat	NSFT (+),	(Oliveira

Methoxyl- diphenyl diselenide	Suppl.		<cortex> (↓) MDA <cortex >	HA (+)	Splash test (+)	et al., 2016)
PSAP	Selenium Suppl.	Mou se	(↓) MAO-A activity, MDA, nitrite <cortex, hippocampus>	Heat HA (+)	FST (+)	(Sousa et al., 2018)
(m-CF ₃ - PhSe) ₂	Selenium Suppl.	Mou se	(↓) TBARS, 4- Hydroxynonenal, PARP <hippocampus>	Heat HA (+)	FST (+)	(Schossler Garcia et al., 2021b)
Curcumin	Curcuma longa constituent	Rat	(↑) NA, 5-HT, DA <cortex, hippocampus> (↓) Substance P, IL-1β, TNF-α, NF-κβ <cortex, hippocampus>	MA (+), Mech. HA (+)	FST (+)	(Arora et al., 2011)
Imperatorin	Coumarin	Mou se	(↓) NMDA-R, NFκB <brain, spinal cord>	MA (+), Mech. HA (+)	FST (+), EPM (+), PAT (+), MWM (+), LA (+)	(Kaur et al., 2019)
Esculetin, Skimmetin Daphnetin	Coumarins	Mou se	(↑) NA, 5-HT, GSH <brain> (↓) Glutamate, IL-1β, TNF-α, TBARS, MAO-A activity <brain>	MA (+), Mech. HA (+)	FST (+), MWM (+)	(Singh et al., 2020; Singh et al., 2021b; Singh et al., 2021c)
Gentiopicroside	Secoiridoid from Gentiana lutea	Mou se	(↑) NA, 5-HT, DA <amygdala> (↓) GluN2B- containing NMDA-R, Caspase-3 <amygdala>	MA (+), Heat HA (+)	FST (+), OFT (+)	(Liu et al., 2014)
β-carotene	Plant carotenoid	Rat	(↑) NA, 5-HT, DA, GSH <cortex> (↓) MDA, H ₂ O ₂ , NO <cortex>	Mech. HA (+)	-	(Elkholy et al., 2021)

Fisetin	Plant polyphenol	Rat	(↑) 5-HT, SOD, GSH <cortex, thalamus, spinal cord> (↓) ROS, MDA <cortex, thalamus, spinal cord>	Mech. HA (+)	FST (+)	(Yao et al., 2020)
Resveratrol	Plant polyphenol	Mouse	(↓) Reactive species <CSF>	MA (+), Heat HA (+)	FST (+)	(Peres Klein et al., 2016)
Aegeline	Alkaloid in Aegle marmelos	Mouse	(↓) iNOS <brain, spinal cord> (↑) GSH <brain> (↓) MAO-A, TBARS <brain>	MA (+), Mech. HA (+)	FST (+)	(Singh et al., 2021a)
Angelica archangelica root extract		Mouse	(↓) Oxidative stress <brain>	Mech. HA (+)	PAT (+), MWM (+), LA (+)	(Kaur et al., 2020)
Ferulic acid	Phytochemical in fruits and vegetables	Mouse	(↑) NA, 5-HT, DA, GSH, SOD <cortex, hippocampus> (↓) LPO, Nitrite, IL-1 β , TNF- α , Substance P, NF- κ B p65, Caspase-3 <cortex, hippocampus>	MA (+), Heat HA (+)	FST (+), TST (+)	(Xu et al., 2013)
Echinocystic acid	Natural triterpene in herbs	Mouse	(↑) NA, 5-HT, DA, 5-HT _{1A} -R <hippocampus> (↓) GluN2B-containing NMDA-R, 5-HT _{2A} -R, phospho-AMPA-R, PSD-95, CaMKII, Caspase-3, iNOS <hippocampus>	Mech. HA (+)	FST (+), TST (+), OFT (+)	(Li et al., 2016)
Melatonin	Pineal gland	Rat	(↑) SOD1, CAT, PGC-1 α ,	-	Wheel	(Favero et

	hormone Suppl.		Mfn2, CoQ10 <gastrocnemius muscle> (↓) Thiol, NLRP3 <gastrocnemius muscle>		running (+)	al., 2017; Favero et al., 2019)
Melatonin/ folic acid	Pineal gland hormone and B vitamin Suppl.	Rat	(↑) SOD, SOD, CAT <brain> (↓) TBARS, LPO, Nitrotyrosine, PARP, microglia activity <brain> (↓) VEGF, NGF, Mast cell infiltration <brain, sciatic nerve>	MA (+), Heat HA (+)	FST (+)	(Fusco et al., 2019)
CoQ10	Antioxidant coenzyme Suppl.	Mou se	(↓) ROS <spinal cord, skeletal muscle>	MA (+), CA (+)	FST (+), Thigmotaxis (+)	(Brum et al., 2020)
AT-RvD1, AT-RvD2	Analogues of D series Rvs	Mou se	(↑) 5-HT <total brain, thalamus> (↑) DA <cortex> (↓) Glutamate <total brain, spinal cord>	MA (+), Heat HA (+)	FST (+)	(Klein et al., 2014)
Acupunctu re	Electro- acupuncture	Rat	(↑) 5-HT <dorsal raphe nucleus>	MA (+)	EZM (+), OFT (+)	(Wu et al., 2015) (Wu et al., 2017)
Exercise	Free gait in shallow water	Mou se	-	Heat HA (-), Electrical stimuli (-)	LA (+)	(Nakata et al., 2020)
Exercise	Aerobic/stren gth trainings	Mou se	(↓) Unmyelinated fibers <DRG>	MA (+), Cold HS (+)	Facial expression (+), TST (+), LA (+)	(Ferrarini et al., 2021)

(↑): Increase, (↓): Decrease, (+): significantly effective compared to vehicle, (-): not significantly effective compared

to vehicle, -: not assessed, Mech.: mechanical, Suppl.: supplementation

Footnote

No author has an actual or perceived conflict of interest with the contents of this article.

This manuscript has not been published before in whole or in part.

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