

REVIEW

Animal models of fibromyalgia

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Abstract

Animal models of disease states are valuable tools for developing new treatments and investigating underlying mechanisms. They should mimic the symptoms and pathology of the disease and importantly be predictive of effective treatments. Fibromyalgia is characterized by chronic widespread pain with associated co-morbid symptoms that include fatigue, depression, anxiety and sleep dysfunction. In this review, we present different animal models that mimic the signs and symptoms of fibromyalgia. These models are induced by a wide variety of methods that include repeated muscle insults, depletion of biogenic amines, and stress. All potential models produce widespread and long-lasting hyperalgesia without overt peripheral tissue damage and thus mimic the clinical presentation of fibromyalgia. We describe the methods for induction of the model, pathophysiological mechanisms for each model, and treatment profiles.

Introduction

Chronic pain is an abnormal and non-protective response that represents a significant health problem affecting over 100 million Americans - more than diabetes, cancer, and heart disease combined [1]. It has been defined as pain that outlasts normal tissue healing time or pain that lasts longer than six months. Approximately 14% of the US population suffers from chronic widespread muscle pain conditions such as fibromyalgia (FMS) [2]. FMS is characterized by widespread pain, which includes the trunk, widespread tenderness to pressure stimuli, and morning stiffness. FMS is also associated with a number of other symptoms, including pronounced fatigue, sleep disturbances, and psychological disturbances (depression and/or anxiety) [3]. The prevalence of co-morbid symptoms varies across the

population, with pain and fatigue occurring in up to 100% of the population, sleep disturbances in 90% and depression or anxiety occurring in 40% [3].

While the underlying cause of FMS is unknown, it has become increasingly clear that a number of systems are altered in people with FMS. Several hypotheses have been proposed as the underlying pathophysiology of FMS: muscular dysfunction, central sensitization, alterations in the hypothalamic-pituitary-adrenal (HPA) axis, and deficits in endogenous pain-modulating systems [4].

Currently patients are managed in a multidisciplinary approach, but are rarely cured. Therefore, it is imperative that a greater understanding of potential causes and pathology in FMS be investigated to guide development of new therapeutics and enhance current treatment strategies. A series of basic pathological alterations have been shown in human subjects. Enhanced cortisol responses and abnormal growth hormone regulation implicate the HPA axis [4]. Reduced serotonin, increased substance P and increased nerve growth factor found in the cerebrospinal fluid of patients with FMS suggest alterations in inhibitory and excitatory neurotransmitters in the central nervous system [3,4]. Enhanced central amplification and reduced central inhibition of pain [3,4] implicate alterations in the central neural response to pain. Additionally, there is a strong familial aggregation for FMS, and evidence for polymorphisms of genes in the serotonergic, dopaminergic and catecholaminergic systems [3,4]. Considering a number of multiple changes in different organic systems, it has been suggested that several factors contribute to FMS, which may be a manifestation of multiple syndromes with similar symptoms. The development of an animal model mimicking FMS is therefore difficult, though the use of animal models are substantially important to gain a better understanding of the development and maintenance of FMS and guide the development of new therapeutics.

Animal models of disease states are valuable tools for developing new treatments and investigating underlying mechanisms. They should mimic the symptoms and pathology of the disease and importantly be predictive of effective treatments. FMS is a differentiated pain

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syndrome as it is diagnosed by symptoms, not by pathological conditions. Thus, an animal model of FMS ideally should include widespread pain and the associated symptoms.

In this review, we present different animal models that mimic the signs and symptoms of FMS. These models produce widespread and long-lasting hyperalgesia without overt peripheral tissue damage and thus mimic the clinical presentation of FMS. For example, several of these models use multiple low-intensity insults to induce the widespread hyperalgesia (for example, multiple acid injections, fatigue with acid injections, hyperalgesic priming), while another uses disruption of biogenic amines in the central nervous system mimicking underlying changes observed in FMS. Lastly, several use unavoidable stress, a known trigger in people with FMS.

Challenges for developing fibromyalgia-like animal models

Unlike animal models for nociceptive and neuropathic pain, which are relatively easy to mimic etiologies, FMS does not have a well-established animal model. The development of an animal model of central (non-nociceptive) pain is somewhat difficult as its etiology is still unknown. Therefore, the models mimic the symptomatology and management profile of the disease. The lack of tissue injury is an important feature in FMS and should be mimicked in animal models of FMS. Further, given the correlation between co-morbidities (fatigue, depression, anxiety) and pain in FMS, corresponding animal models should ideally simulate the development of these symptoms. Lastly, given that FMS predominantly occurs in women, studies should be performed in female animals, and compare males and females. Sex differences are likely to be important in the development as well as the maintenance of FMS-like symptoms. Despite these challenges, few animal models have been tested for co-morbidities and most models have been tested in males. Table 1 summarizes the literature for individual models.

Fibromyalgia-like animal models

The models outlined below primarily develop in response to repeated stimuli applied to the muscle, or stress combined with nociceptive stimuli applied to the muscle. In fact, these stimuli are often below threshold or produce a short lived response hyperalgesia in the animal, but when combined can produce a much longer-lasting hyperalgesia. While the duration of the hyperalgesia varies between models, and in some cases between sexes, the common theme of repeated insults to the organism remains. The combination of repeated insults is time-dependent with an 'ideal' window separating the multiple insults for the hyperalgesia to develop [5,39]. It is also clear that, in some of the models, the

noxious stimuli can be given in distinctly different regions of the body and still produce hyperalgesia [9,39]. Combinations of stress along with muscle insult can prolong the duration of hyperalgesia. How this relates to the etiology of FMS is not entirely clear, but it may be that a combination of multiple stressors to the organism is necessary for widespread, long-lasting hyperalgesia to occur. These stressors could be repeated low-intensity localized insults, or could be more widespread and include conditions such as a sedentary lifestyle, stress, fatigue, obesity, or sex. These factors can result in molecular and cellular changes in both nociceptors and in central neurons that result in sensitization to maintain the pain. Further, the peripheral and central pathways can interact to further enhance the observed molecular and cellular changes to enhance the pain. Future studies will be required to translate these findings from animal models to human subjects.

Repeated muscle insult models: priming

Acid saline-induced pain model

Description

The non-inflammatory pain model induced by repeated intramuscular acid injections in rodents has been suggested to have face validity to chronic widespread pain conditions (such as FMS) in humans. In this model, two injections of acid saline (pH 4.0) separated by 2 to 5 days, given unilaterally into the gastrocnemius muscle, produce a bilateral decrease in hindpaw and muscle mechanical withdrawal thresholds that lasts for 4 weeks (male and female rats and mice) [5,24,25]. Further, there is enhanced visceral (secondary) hyperalgesia and reduced physical activity (male rats and mice) [6,7]. Administering the first acid injection into one gastrocnemius muscle and the second to the contralateral gastrocnemius muscle produces comparable hyperalgesia to that produced by both acid injections in the same muscle (male rats) [9]. There is also modulation of the cardiac autonomic system after the development of mechanical hyperalgesia, shifting the autonomic balance towards a sympathetic predominance and reduction in baroreceptor reflex sensitivity (male rats) [12]. This experimental pre-clinical finding reinforces the hypothesis that different organic systems present simultaneous dysfunctions in FMS.

In this model hyperalgesia develops similarly between male and female mice [10], and shows similar results between rats and mice [5,10]. Morphological analysis of the injected muscle reveals no obvious muscle damage or inflammation associated with acidic saline injections, and removal of afferent input from the injected site has no effect on the contralateral hypersensitivity (male rats) [5]. On the other hand, the hypersensitivity once

Table 1 Main findings of different animal models of fibromyalgia according to central and peripheral factors

Model	Main findings	
	Peripheral	Central
Repeated muscle insult		
Acid saline induced pain	No peripheral damage [5]	Widespread (visceral, cutaneous and muscle) and long-lasting mechanical hyperalgesia [5,6]; decreased voluntary physical activity [7]
	Reversal of mechanical hyperalgesia by neurotrophin-3 [8]	Muscle hyperalgesia is similarly developed if the first acid injection is performed in different muscles, such as right lateral gastrocnemius, right medial gastrocnemius or left lateral gastrocnemius, keeping the second injection at the same site for all [9]
	ASIC3 ^{-/-} mice do not develop hyperalgesia [10]	ASIC3 ^{-/-} mice do not develop hyperalgesia or central neuron sensitization, localization of ASIC3 in muscle afferents [10]
	Substance P mediates NK1 receptor pathway to block acid activation in muscle nociceptors [11]	Shift of the cardiac autonomic balance towards a sympathetic predominance and reduction in baroreceptor reflex sensitivity [12]
	Aerobic exercise with treadmill running reduces cutaneous and muscle hyperalgesia and increases neurotrophin-3 levels in muscle [13]	Increased ERK activity in the amygdala, and increased post-synaptic excitatory transmission from parabrachial nucleus in amygdala [14]
		T-type calcium channels mediate hyperalgesia supraspinally, increases in ERK in paraventricular thalamus that depend on activation of T-type calcium channels [15]
		Local anesthetic in the RVM during the second injection prevents the development of muscle hyperalgesia and reverses existing hyperalgesia [16]
		Increased release of glutamate and aspartate and decreased glycine in the RVM during the second injection [17]
		Reversal of both cutaneous and muscle hyperalgesia through microinjection of NMDA receptor antagonists into the RVM, and only cutaneous hyperalgesia into the NGC [18]
		Increased expression of NR1 subunit of NMDA receptor in the RVM produces muscle hyperalgesia; downregulation of NR1 subunit in RVM prevents development of muscle hyperalgesia [19]
		Reversal and prevention of hyperalgesia by blockade of spinal NMDA and non-NMDA glutamate receptors [20]
		Increased number of spinothalamic neurons expressing p-NR1 in lamina X of the spinal cord [21]
		Increased number of spinothalamic neurons expressing p-NR1 in lamina X of the spinal cord [21]
		Increased spinal glutamate and aspartate concentrations in the spinal cord in a calcium-dependent manner [22]
	Phosphorylation of CREB through activation of the spinal cAMP pathway in a time-dependent manner [23]	
	Muscle stimulation produces Fos expression ipsilaterally in all regions of the dorsal horn; paw stimulation produces ipsilateral Fos expression in the superficial spinal laminae, and bilaterally in deep laminae [24]	
	Reversal of mechanical hyperalgesia by μ and δ opioid receptor agonist (but not κ), glutamate receptor antagonist, pregabalin, reuptake inhibitors, K ⁺ channel opener, Na ⁺ channel blocker, but not cyclooxygenase-2	

Table 1 Main findings of different animal models of fibromyalgia according to central and peripheral factors
 (Continued)

		inhibitor and benzodiazepine; co-administration of tramadol and milnacipran is more effective in reducing muscle hyperalgesia than tramadol alone [20,25-28]
		Low intensity exercise reduces mechanical hyperalgesia, which is attenuated by opioid receptor agonist [29]; regular exercises prevent the development of muscle pain and exercise-induced chronic muscle pain through the decrease of phosphorylation of the NR1 in the RVM [30]
Hyperalgesic priming	<p>Development of chronic muscle hyperalgesia (2 weeks) after acute muscle inflammation [31]</p> <p>Priming hyperalgesia (cutaneous) involves activation of a cAMP/PKCε in nociceptors [31,32]</p> <p>IL-6 in muscle primes response to subsequent PGE2, and antisense to IL-6 or gp130 prevents hyperalgesic priming [33]</p> <p>Spinal pretreatment with oligodeoxynucleotide antisense to PKCε reduces muscle hyperalgesia [31]</p> <p>Cutaneous inflammation primes the hyperalgesic response to a subsequent injection of PGE2, 5-HT, or A2 agonist; PKCε inhibitor prevents priming effect [34]</p> <p>αCaMKII produces hyperalgesic priming; inhibition of αCaMKII prevents activation of PKCε-induced priming; activation of αCaMKII produces priming that is not prevented by pretreatment with PKCε antisense [35]</p> <p>Inhibitors of enzymes implicated in the metabolism of cyclic nucleotides to adenosine; A1 adenosine receptors block late phase of PGE2-induced muscle hyperalgesia [36]</p> <p>Pretreatment with a selective neurotoxin (IB4-saporin) prevents GDNF-induced hyperalgesia; NGF and PsiepsilonRACK produce muscle hyperalgesia [37]</p> <p>Hyperalgesic priming is reversed through the inhibition of translation in the peripheral terminal of the nociceptor [38]</p>	
Fatigue enhanced muscle pain	<p>No inflammation or damage to muscle with whole-body or single-muscle fatigue tasks; no change in muscle lactate, pCO₂, pO₂, creatinine kinase, or phosphate after whole-body muscle fatigue [39,40]</p> <p>Reduced muscle force after whole-body fatigue or single-muscle fatigue task [39,40]</p>	<p>Whole-body fatigue enhances hyperalgesia to muscle insult [40,41]; single muscle fatigue enhances hyperalgesia to muscle insult [39]</p> <p>Enhancement of paw and muscle hypersensitivity in both male and female mice [39,41]</p> <p>Female mice show greater enhancement of hyperalgesia that is prevented by ovariectomy (whole body fatigue); greater time-window, greater spatial window for induction of hyperalgesia, and longer lasting hyperalgesia not affected by ovariectomy (single-muscle fatigue) [39,41]</p> <p>Blockade of RVM NMDA receptors during fatigue prevents development of hyperalgesia [42]</p> <p>c-Fos expression in nucleus raphe pallidus, obscurus, and magnus; increased p-NR1 in RVM with whole-body fatigue but not single-muscle fatigue [30,39,42]</p> <p>Prevention of hyperalgesia and enhanced p-NR1 in RVM by regular physical activity [30]</p>
Biogenic amine depletion		<p>Reduction of levels of biogenic amines in central nervous system [43]</p> <p>Increased time of immobility as a measure of depression [43]</p> <p>Enhanced sensitivity to muscle and cold stimuli after reserpine; no pathology in peripheral nerves or brain [44]</p>

Table 1 Main findings of different animal models of fibromyalgia according to central and peripheral factors
 (Continued)

		Depletion of biogenic amines with reserpine leads to development of muscle hyperalgesia that is reversed by pregabalin, duloxetine, and pramipexol, but not diclofenac; 5HT _{2C} receptor agonists reverse muscle hyperalgesia induced by reserpine; NSAIDs have no effect [43,45]
Stress		
Cold stress		<p>Bilateral muscle and paw mechanical hyperalgesia, thermal hyperalgesia [46,47]</p> <p>Increased plasma corticosterone concentration; no change in anxiety or depression-like behaviors [46]</p> <p>Decreased serotonin and its metabolites in brain and spinal cord after repeated cold stress [48]</p> <p>Decreased morphine analgesia; increased U50,488 analgesia; decreased DAMGO analgesia when given supraspinally; diazepam prevented the decreased DAMGO analgesia [49]</p> <p>Post-translational changes in proteins supraspinally after stress [50]</p> <p>Blockade of substance P and calcitonin gene-related peptide, NMDA receptors in spinal cord reduces cold-stress hyperalgesia [51-53]</p> <p>Hyperalgesia reversed by gabapentin and antidepressants [46,54]</p>
Sound stress	<p>Increase in hyperalgesia after local injections of PGE₂, epinephrine, or LPS [55,56]</p> <p>Increased plasma levels of epinephrine and increased activity of catecholamine synthesizing enzymes in adrenal medulla for >28 days [55,58]</p> <p>IL-6 downregulation on primary afferents prevents hyperalgesia dependent on glucocorticoids and catecholamines [56]</p>	<p>Widespread hyperalgesia of paw, viscera and jaw muscle [57]</p> <p>Increased anxiety [57]</p>
Subchronic swimming stress		<p>Thermal hyperalgesia, decreased grip force, enhanced response to inflammatory irritants [59-61]</p> <p>Enhanced c-fos expression in response to formalin spinally [60]</p> <p>Basal and evoked release of GABA is decreased, and glutamate is increased, in spinal cord [62,63]</p> <p>Reversal of hyperalgesia by reuptake inhibitors, serotonin precursor tryptophan, and diazepam [59,61,62]</p>

αCaMKII, α calmodulin-dependent protein kinase II; ASIC, acid sensing ion channel; DAMGO, [D-Ala², N-MePhe⁴, Gly-ol]-enkephalin; GDNF, glial cell line-derived neurotrophic factor; LPS, lipopolysaccharide; NGC, nucleus gigantocellularis; NGF, nerve growth factor; NK, neurokinin; NSAID, non-steroidal anti-inflammatory drug; PEG₂, prostaglandin E₂; PKC, protein kinase C; RVM, rostroventral medial medulla.

developed is reversed by blockade of excitatory activity spinally or supraspinally (male rats) [16,18,20,23]. Together, these data suggest that once developed, this model is maintained primarily by central mechanisms and is independent of a nociceptive drive. It further mimics the clinical presentation of signs and symptoms observed in FMS with widespread hyperalgesia, minimal muscle tissue damage, alterations in central nociceptive processing, and alterations in cardiovascular autonomic balance.

Pathophysiology

Induction of hyperalgesia

Peripheral, spinal and supraspinal mechanisms are all involved in the induction of the hyperalgesia in this repeated acid model. Induction of the model requires activation of acid sensing ion channel (ASIC)₃ in muscle afferents as ASIC₃^{-/-} mice do not develop hyperalgesia (male and female) [10], suggesting activation of muscle afferents initiates the hyperalgesia. In human subjects,

infusion of acidic buffer into the muscle produces pain and hyperalgesia, with females being more likely to develop referred pain than males [64,65].

Neurotrophin (NT)-3 overexpression in muscle prevents chronic secondary hyperalgesia induced by acid saline injection (male and female mice) [8]. Further, spinal increases in *c-fos* after repeated acid injection are prevented by NT-3 overexpression [8], further supporting that initial activation of muscle afferents drives central changes.

Interestingly, muscle hyperalgesia is produced by a single acid injection in mice with a deletion of the tachykinin precursor 1 (*Tac1*) gene or those given a co-administration of neurokinin 1 (NK1) receptor antagonists at the time of induction, suggesting that substance P may play a role in inhibiting the development of widespread pain [11]. Substance P inhibits acid activation of ASIC3-muscle nociceptors and this may play a role in regulating development of chronic widespread pain [11].

Central mechanisms involving the spinal cord, brainstem and cortex have also been implicated in the development of hyperalgesia in this repeated acid model. Development of acid-induced hyperalgesia can be prevented by blockade of neuronal activity in the rostroventral medial medulla (RVM), a site known to facilitate nociception, during the second acidic saline injection, but not during the first, suggesting alterations in the RVM in response to the first injection (male rats) [16]. Indeed, during the second acidic saline injection, excitatory neurotransmitter release increases and inhibitory neurotransmitter release decreases in the RVM (male rats) [17]. Thus, alterations in excitatory and inhibitory balance in the RVM could potentially trigger development of widespread hyperalgesia.

Spinally, development of hyperalgesia is delayed by blockade of NMDA-glutamate receptors during the second injection but not the first (male rats) [20]. In parallel there is an increase in release of the excitatory neurotransmitter glutamate in the spinal cord during the second but not the first acid injection (male rats) [22]. Similar to the RVM, glycine is decreased in response to the second acidic saline injection [22]. Thus, as in the RVM, alterations in excitatory and inhibitory balance can trigger development of hyperalgesia.

Little is known about cortical sites in this model despite evidence from imaging studies implicating several cortical areas in abnormal processing of nociceptive input in people with FMS [66]. Recently, however, Cheng and colleagues [14] have investigated cortical and thalamic sites; there are increases in phosphorylation of ERK and there is enhanced postsynaptic excitatory transmission from the parabrachial nucleus in the central nucleus of the amygdala (male and female mice). Increases in phosphorylation of ERK also occur

in the paraventricular thalamus after repeated acid injections (male and female mice) [15]. The hyperalgesia and the increases in phosphorylation of ERK are prevented by intracerebroventricular blockade of T-type Ca^{2+} channels (T-channels) [15]. Conversely, if ERK is not phosphorylated, hyperalgesia is not prevented [15]. Together these data suggest that cortical sites are altered by repeated acid injections. Future studies should continue to examine cortical sites and connections between cortical sites and brainstem sites to gain an understanding of this pain condition.

Maintenance of hyperalgesia

The hyperalgesia in this model appears to be independent of continued afferent input as removal of muscle afferents or blockade of ASICs has no effect on it once developed (male mice and rats) [5,67,68]. Furthermore, expression of ASIC-like currents or their properties in labeled muscle dorsal root ganglion neurons are not altered following repeated acid injections, suggesting that hyperalgesia is not related to changes in ASIC expression in this model (male mice) [67]. Indeed, maintenance of the hyperalgesia once developed involves activation of spinal and supraspinal mechanisms.

In the spinal cord, recordings from nociceptive spinal neurons show enhanced sensitivity to innocuous and noxious mechanical stimuli bilaterally along with a bilateral spread of receptive fields (male and female mice) [10]. Spinally, after development of hyperalgesia there are increases in concentrations of glutamate, increases in phosphorylation of the NR1 subunit of the NMDA receptor in spinothalamic tract neurons, and increases in phosphorylation of the transcription factor CREB (male rats) [21-23], all suggesting increased excitatory activity in the spinal cord. Blockade of NMDA or non-NMDA glutamate receptors, or the cAMP pathway reverse the already developed hyperalgesia (male rats) [20,23]. Interestingly, spinal activation of the protein kinase C (PKC) pathway produces mechanical hyperalgesia that is prevented by blocking PKC, NMDA, or AMPA/kainite receptors. Nonetheless, PKC is not used for maintaining chronic hyperalgesia in this model (male rats) [69].

The RVM appears to be integral to the maintenance of chronic widespread pain after repeated acid injections. Blockade of neuron activity in the RVM reverses the acid-induced muscle hyperalgesia, suggesting the RVM plays a key role in maintaining hyperalgesia once developed (male rats) [16]. NMDA receptors in the RVM play a key role in the hyperalgesia induced by repeated acid injections. Over-expression of the NR1 subunit of the NMDA receptor in the RVM produces hyperalgesia; downregulation of NR1 in the RVM

reduces hyperalgesia; there is enhanced phosphorylation of NR1 in the RVM; and blockade of NMDA receptors in the RVM reverses the existing hyperalgesia (male rats) [18,19].

Thus, repeated intramuscular injections require muscle afferents and central mechanisms for induction of hyperalgesia; and central mechanisms for maintenance. A variety of excitatory and inhibitory neurotransmitters, receptors, and pathways are involved in this process. Future investigations should expand on these studies in the spinal cord and brainstem, and begin to look for changes in cortical areas that process pain.

Treatment effects

This model shows a similar pharmacological management profile to clinical treatment of FMS: reductions in pain and hyperalgesia by antidepressants, anticonvulsants, opioids, glutamate receptor antagonists, K⁺ channel openers, Na⁺ channel blocker and exercise, but not non-steroidal anti-inflammatory drugs (NSAIDs; male rats) [13,25-29,70]. Administration of alosetron (5-HT₃ receptor antagonist), either intravenously or intrathecally, reverses the mechanical hypersensitivity observed after acid saline injections and prevents the development of visceral hyperalgesia (male rats) [70]. Pregabalin (anticonvulsant drug) decreases both cutaneous and deep tissue hyperalgesia (male rats) [25]. Moreover, tramadol (a centrally acting synthetic opioid analgesic used to treat moderate to moderately severe pain) and milnacipran (a serotonin-norepinephrine reuptake inhibitor used in the clinical treatment of FMS) showed a potent antihyperalgesic effect when administered together (male rats) [27]. Taken together, acid-induced hyperalgesia is able to be reversed by a series of analgesic strategies commonly used clinically in FMS.

Exercise is an effective treatment clinically for FMS [71]. Similarly, in the repeated acid model mechanical hyperalgesia is reversed or prevented with different exercise protocols. Low intensity exercise (walking on a treadmill for 5 consecutive days) reverses hyperalgesia in an opioid-dependent manner (male rats) [29]. Moderate-intensity exercise training (walking on a treadmill for 5 days per week for 3 weeks) decreases cutaneous and deep tissue acid saline-induced mechanical hyperalgesia and increases NT-3 in muscle (female mice) [13], suggesting the reduction in mechanical hyperalgesia after exercise may be a result of elevated levels of NT-3 protein. Regular exercise training in running wheels (8 weeks) prevents the development of chronic muscle hyperalgesia in mice (male mice) [72]. In parallel, the increase in phosphorylation of NR1 that normally occurs after repeated acid injections does not occur in animals that were physically active (running wheels) [72]. Thus,

the model is responsive to exercise as well as pharmacological management.

Limitations and future directions

The hyperalgesia that develops in this model is generally considered widespread and includes bilateral hindlimb muscles, paw and viscera [6,25]; however, it is unclear if there are changes in other areas of the body, such as the forelimbs. The model does show reduced activity levels, and develops in sedentary but not physically active animals [7,72] and thus mimics the reduced activity and enhanced prevalence of chronic pain in people with FMS [73,74]. Future studies should confirm the prevention of chronic pain in human populations with acute pain by improving activity levels. It is unclear if there are comorbid symptoms of depression, anxiety, fatigue or sleep disturbances in this model, and future studies should assess these co-morbidities. While the pharmacological profile of this model generally matches that of people with FMS, the model is sensitive to opioids delivered intrathecally [26], which is different from the clinical picture of those with FMS [75]; however, it should be noted that tramadol, a weak opioid agonist combined with a reuptake inhibitor, is effective in people with FMS [76]. Lastly, most studies were performed in male mice or rats despite the fact that FMS occurs more frequently in females; future studies should perform mechanistic studies in both males and females.

Hyperalgesic priming model

Description

Another model with multiple insults uses an acute inflammatory insult or mediator to induce hyperalgesia. After the hyperalgesia resolves, prostaglandin E₂ (PGE₂) injected into the same muscle results in a muscle hyperalgesia that lasts for at least 14 days as compared to hours in controls that did not receive the inflammatory insult (carrageenan or IL-6) (male rats) [31,33]. Similarly, priming of the skin with carrageenan injected intradermally to the hindpaw results in a prolonged response to PGE₂ lasting over 24 hours compared to less than 4 hours in naive animals (male rats) [32,34,37].

Pathophysiology

Induction of hyperalgesia

Peripheral mechanisms have been extensively studied in this model. Specifically, depletion of IB₄(+) nociceptors with the neurotoxin IB₄-saporin prevents the development of the hyperalgesia (male rats) [37]. Further, depletion of the second messenger PKC ϵ (with an oligodeoxynucleotide antisense) also prevents the development of the hyperalgesia (male rats) [31]. Conversely, PsiepsilonRACK, which selectively activates PKC ϵ ,

induces muscle hyperalgesia (male rats) [37]. Inhibiting α calmodulin-dependent protein kinase II prevents activation of PKC ϵ -induced priming (male and female rats) [35]. Thus, these data show that IB4+ nociceptors and PKC ϵ along with α calmodulin-dependent protein kinase II play a significant role in the priming model of muscle hyperalgesia.

Maintenance

The maintenance of the hyperalgesia in this priming model also involves peripheral nociceptors. Hyperalgesic priming is reversed through the inhibition of translation in the peripheral terminal of the nociceptor by injecting either rapamycin or cordycepin into muscle (male rats) [38]. In inflammation-pretreated animals, the late phase of PGE2-induced hyperalgesia, but not the early, was blocked by injecting inhibitors of enzymes that block the metabolism of cyclic nucleotides to adenosine, and by blockade of A1 adenosine receptors (male rats) [36]. Thus, once developed, changes in nociceptors that are related to increased gene production, activation of PKC ϵ and adenosine may maintain the hyperalgesia in the hyperalgesic priming model.

Limitations and future directions

The hyperalgesia that develops in this model lasts for at least 2 weeks after induction [31]; it is unclear how long this hyperalgesia continues. It is clear that the hyperalgesia is longer-lasting than when the same stimuli are applied to the skin [34] and may indicate differential processing of muscle and cutaneous pain either peripherally or centrally. Standard pharmacological and non-pharmacological treatments for FMS, co-morbid symptoms of FMS, and changes in central processing have yet to be studied in this model. Again, most studies were performed in males and future studies should perform experiments in both sexes to ensure mechanisms are similar.

Fatigue-enhanced muscle pain

Description

Combining a muscle fatiguing task with a low-intensity muscle insult results in long-lasting and widespread hyperalgesia. Muscle fatigue has been produced by either having animals run in a running wheel for 2 hours prior to the muscle insult or by direct electrical stimulation of the muscle. The whole-body fatiguing task has been followed by either two intramuscular injections of pH 5.0 saline or one intramuscular injection of 0.03% carrageenan (male and female mice) [40-42]. Long-lasting muscle and cutaneous hyperalgesia develop after the muscle insult combined with whole-body fatigue. There is no muscle damage, no change in muscle lactate, pCO₂, pO₂, creatinine kinase, phosphate, or histology in

whole-body fatigued animals. Further, in this whole-body fatigue model, there is an enhanced hyperalgesia (carrageenan plus fatigue) in female mice compared to male mice that is dependent on estrogen [40].

A single muscle fatigue task, induced by electrical stimulation, in combination with two injections of pH 5.0 saline, also enhances the hyperalgesia in a sex-dependent manner (male and female mice) [39]. Specifically, male and female mice, fatigued immediately prior to muscle insult in the same muscle, develop similar muscle hyperalgesia 24 hours later. However, female mice develop hyperalgesia contralaterally and the hyperalgesia lasts longer. Further hyperalgesia is easier to induce in female mice: females develop hyperalgesia when the muscle fatigue and muscle insult occur in different muscles, and when muscle insult is administered 24 hours after fatigue. The muscle insult with or without muscle fatigue results in minimal inflammatory changes in the muscle itself, and sex differences are not related to estradiol (ovariectomy) or changes in brainstem activity (pNR1) [39].

Pathophysiology

For the whole-body fatigue task, there is increased activation of neurons in the brainstem RVM: nucleus raphe pallidus, obscurus, and magnus as measured by c-fos expression in response to the fatigue task. Further, there is increased phosphorylation of the NR1 subunit of the NMDA receptor in the RVM after the whole-body fatigue task combined with muscle insult (male and female mice) [42,72], and blockade of NMDA receptors in the RVM during the fatigue task prevents the development of the hyperalgesia (male and female mice) [42]. In contrast, the single-muscle fatigue task combined with muscle insult has no effect on the phosphorylation of NR1 in the RVM (male and female mice) [39]. It appears that the whole-body fatigue model involves central changes in the brainstem in sites involved in both pain and fatigue while the single muscle fatigue model may involve different mechanisms. Future studies are needed to understand in more detail the underlying mechanisms of these models.

Limitations and future directions

The hyperalgesia that develops in the fatigue-induced models lasts for between 7 days (males) and 35 days (females) [31]. It is clear that muscle fatigue can enhance the hyperalgesia produced by low-intensity muscle insult, as observed in people with FMS [77]. However, how this muscle fatigue relates to symptoms of fatigue in people with FMS is unclear. Standard pharmacological and non-pharmacological treatments

for FMS and co-morbid symptoms of FMS are yet to be determined.

Biogenic amine depletion model

Description and pathophysiology

For this animal model, biogenic amines are depleted systemically by reserpine to mimic the alterations in biogenic amines observed clinically in FMS (male rats) [43-45]. Repeated administration of reserpine (1 mg/kg subcutaneously, once daily, for three consecutive days) causes long-lasting widespread muscle and cutaneous hyperalgesia that is sustained for at least 1 week in both male and female rats. This treatment regimen decreases the amount of biogenic amines (dopamine, norepinephrine, and 5-hydroxytryptamine) in the spinal cord, thalamus, and prefrontal cortex. There is also an increase in immobility time in the forced swim test, a test for depression, which is a common co-morbid symptom of FMS (male rats) [43].

Treatment effects

Reserpine-induced mechanical hyperalgesia is reduced by administration of anticonvulsants, serotonin-norepinephrine reuptake inhibitors, and dopamine agonist drugs, but not by NSAIDs. Lorcaserin, YM-348 and vabicaserin, all 5HT_{2C} receptor agonists, also reverse the hyperalgesia induced by reserpine (male rats) [45]. The lack of NSAID-induced analgesia, and the modulation amines, matches the pharmacological profile for managing chronic pain in FMS [78,79].

Limitations and future directions

While this model has been characterized pharmacologically, and animals show signs of the co-morbid symptom of depression, future studies are needed to determine how alterations of the serotonergic system contribute to the development and maintenance of hyperalgesia. Further, all the studies so far have been performed in males and future studies should confirm these effects in females and examine if there are differences in the mechanisms between males and females.

Stress models

Cold stress

Description

Intermittent cold stress (ICS), also referred to as repeated cold stress, is used as a procedure for a mouse model that mimics symptoms of chronic widespread pain. In the ICS model, mice are kept in a cold room (-3 to +4°C) overnight for 3 days, and transferred between normal room temperature (24°C) and a cold room every 30 minutes during the day (male rats) [46]. Muscle hyperalgesia lasts at least 3 weeks after repeated cold stress and was longer

and more severe after stress at -3°C than at 4°C. Cutaneous hyperalgesia was observed after stress at -3°C, but not at 4°C (male rats) [47]. This model has several advantages, as no specific apparatus, except for a conventional refrigerator, is necessary, and the short period of stress application (three nights) is sufficient to cause hyperalgesia. While plasma corticosterone concentration is increased after ICS stimulus, anxiety and depression-like behaviors do not develop [46].

Pathophysiology

Supraspinal and spinal nociceptive processing is altered in the cold stress-induced myalgia [47]. Supraspinally, an ICS-induced impairment in the descending inhibitory system has been reported. There is a reduction of both serotonin (5-HT) and 5-hydroxy indoleacetic acid (5-HIAA) levels in supraspinal regions, such as hypothalamus, thalamus, midbrain, and pons plus medulla oblongata, in repeatedly cold-stressed rats (male rats) [48]. ICS-induced hyperalgesia is reduced by spinal blockade of substance P, calcitonin-gene-related peptide, NMDA-glutamate receptors and neurokinin-1 receptors (male rats) [51-53]. Further, there are alterations in the opioid system in the spinal cord in ICS-treated rats with decreases in mu-opioid agonist antinociception, and increases in antinociception by kappa-opioid receptors (male mice) [49]. In addition, post-translational changes in proteins have been observed supraspinally after ICS (male rats) [50]. Thus, spinal and supraspinal changes in both excitatory and inhibitory systems are observed and may underlie the development and maintenance of the hyperalgesia in this model.

Treatment effects

Anticonvulsant and antidepressant drugs reduce ICS-induced hyperalgesia. Specifically, systemic gabapentin reverses hyperalgesia at the one-tenth dose for neuropathic pain-induced rats, and central gabapentin produced long-lasting analgesia (4 to 5 days) in the stress-induced hyperalgesia model, but not in the neuropathic pain model (male and female mice) [54]. A single or repeated intrathecal administration of the antidepressant (milnacipran, amitriptyline, mianserin or paroxetine) reduces hyperalgesia (male mice) [46]. Morphine produces analgesia in ICS mice when injected spinally or peripherally, but not systemically or supraspinally, suggesting that supraspinal sites do not contribute to the analgesia of morphine in this model (male mice) [80]. One hypothesis proposed by the authors is that the lack of morphine-induced analgesia supraspinally could be explained by a loss of descending pain-inhibitory activation, as periaqueductal gray-RVM-spinal cord

interconnection areas are important sites for morphine analgesia (male mice) [80].

Limitations and future directions

The hyperalgesia that develops in this model lasts for at least 3 weeks after induction [31]; it is unclear how long this hyperalgesia continues. Standard pharmacological agents effective in FMS are also effective in this model; however, differing from FMS [75] opioids reduce the hyperalgesia in the cold-stress model. Co-morbid anxiety and depressive-like behaviors do not develop in this model, differing from people with FMS; other co-morbid symptoms have not been tested. Again, most studies have been performed in males and future studies should include females and confirm the mechanisms are similar between males and females.

Sound stress model

Description

In this model, animals are exposed to sound stress over 4 days. Animals are placed in a cage placed 25 cm from a speaker that emits 4 pure tones (5, 11, 15, and 19 kHz). Amplitudes vary through time independently from 20 to 110 dB level at random times each minute, lasting 5 or 10 seconds. Animals are exposed to the sound stressor on days 1, 3, and 4 (male rats) [55]. Sound stress by itself has no effect on mechanical sensitivity in rats. However, there is an increase and more prolonged cutaneous hyperalgesia in response to local injections of PGE2 or epinephrine [55]. There is also an increase in visceral sensitivity and hyperalgesia of the masseter muscle after intraplantar PGE2 in animals exposed to sound stress, showing the widespread nature of the hyperalgesia (male rats) [57]. Further, animals show increases in the anxiety index on the elevated plus maze, suggesting animals show a co-morbid anxiety [57]. Together these symptoms of widespread hyperalgesia (paw, viscera, jaw) and anxiety mimic clinical symptoms and co-morbidities in FMS patients who have widespread pain, and a higher incidence of irritable bowel syndrome, temporomandibular disorder, and anxiety.

Pathophysiology

The repeated exposure to sound stress leads to long-lasting increased activity of catecholamine synthesizing enzymes in the adrenal medulla (male rats) [55,58] that results in increased plasma levels of epinephrine for at least 28 days after the last exposure to sound stress [55], suggesting that a long-lasting stress-induced alteration in the animal persists well beyond exposure to the starting stress factor. After sound stress, lipopolysaccharide injected into skeletal muscle results in long-lasting mechanical hyperalgesia. Treatment with antisense oligodeoxynucleotide to decrease expression of IL-6 receptor

on primary afferent neurons blocked lipopolysaccharide-induced hyperalgesia in stressed rats, but did not attenuate it in non-stressed rats (male rats) [56]. Thus, alterations in cytokines and the HPA axis may underlie stress-induced enhancement of hyperalgesia.

Limitations and future directions

The duration of hyperalgesia and the responsiveness of the hyperalgesia to clinical pharmacological agents used to treat FMS should help to characterize and validate the model. Co-morbid anxiety develops in this model and future studies could confirm other co-morbid symptoms like depression, fatigue, or sleep disturbances. Again the studies were performed only in male rats and thus future studies should include females and confirm mechanisms are similar between males and females.

Subchronic swim stress

In the subchronic swim stress model, rats are forced to swim in water at room temperature for 10 to 20 minutes per day for 3 days (male rats) [59]. Animals develop hyperalgesia to thermal stimulus (hot plate), decreases in grip force lasting for up to 9 days and enhanced response to inflammatory stimuli (formalin and carrageenan) (male rats) [59-61]. Changes in the spinal cord have been observed in this model with enhanced c-fos expression in response to formalin, decreased basal and evoked release of the inhibitory neurotransmitter GABA and enhanced basal and evoked release of glutamate (male rats) [60,62,63], suggesting both enhanced central excitability and reduced central inhibition. Pharmacological validation of the model has been done by showing efficacy to reuptake inhibitors (clomipramine, fluoxetine, milnacipran), the serotonin precursor tryptophan, and diazepam (male rats) [59,61,62].

Limitations and future directions

This model has only begun to be characterized and future studies can begin to examine underlying mechanisms. It is unclear if there is enhanced widespread hyperalgesia to mechanical stimuli as well as thermal stimuli, if there are alterations in peripheral and supraspinal nociceptive pathways, if there are sex differences in the induction or presentation of the model, or if there are co-morbid symptoms.

Other emerging stress models

Other models combining stress with a peripheral insult have recently emerged and may prove useful in the study of FMS. These include the restraint-stress model where rats are restrained in plexiglass tubes (1 hour per day, daily for 6 weeks) that eliminate movement (male rats) [81]. These animals show signs of hyperalgesia to mechanical, heat and cold stimuli within a

week that remains through the 6 weeks of stress, and enhanced response to formalin [81]. Using ultrasonic vocalization as a sign of stress, rats were subjected to unavoidable scrambled foot shock-light stimuli. In this model there is enhanced response to formalin that is reduced by pregabalin and reuptake inhibitors (male rats) [82].

Perspectives

The current available animal models of chronic widespread pain have been induced by different means, including repeated muscle insults (priming techniques), depletion of biogenic amines, and stress with or without muscle insult. Similarities in presentation with widespread hyperalgesia and minimal peripheral tissue damage are found in nearly every model. However, there are clear peripheral and central nervous system changes that may be dependent on the individual model. These models are all relatively new and are currently being explored in more detail. Several questions arise from each of these models and there are some central themes to each. Which models result in peripheral sensitization? Which models result in central sensitization? Is there a continued afferent driver in some conditions? Is there a central driver in others? Are excitatory neurotransmitters, such as substance P, glutamate, or neurotrophic factors, altered peripherally or centrally? Is there involvement in other systems such as the immune system, endocrine system, or the hypothalamic-pituitary axis? How does each of these models differ and how are they similar? Do these models show a similar pharmacological and non-pharmacological treatment profile to people with chronic widespread pain? Will these models be predictive of future treatments and be useful for drug development?

So far, studies on animal models of FMS included in this review have essentially focused on sensory changes such as mechanical and thermal hyperalgesia for characterizing those models. This is an excellent start and completely comprehensible as widespread pain is the main symptom in FMS [83]. However, effects on physical activity levels, quality of life and non-reflexive behavioral tests should also be considered. In addition, co-morbid symptoms such as fatigue or sleep disturbances, as well as emotional dysfunction, including anxiety, depression and mood should be investigated. Interestingly, while FMS is predominately a female disease, most studies were done on males. Future studies should at minimum include female mice in the dataset and examine for potential sexual dimorphisms.

Conclusions

The animal models reviewed in this manuscript are diverse in terms of induction, and diverse in terms of

potential underlying mechanisms. No one model is likely the 'ideal' or 'best' model to mimic FMS. As FMS is also a diverse syndrome that likely has multiple etiologies and multiple subtypes, these models may be useful to ascertain particular pathways and mechanisms that could be altered to result in the manifestation of chronic widespread pain. A mixture of diverse animal models of FMS, each mimicking particular biomarkers and clinical conditions observed in FMS, may contribute to the comprehension of its pathophysiology and improvement of its management.

Note: This article is part of the series on *New perspectives in fibromyalgia*, edited by Daniel Clauw. Other articles in this series can be found at <http://arthritis-research.com/series/fibromyalgia>

Abbreviations

ASIC: Acid sensing ion channel; FMS: Fibromyalgia; HPA: Hypothalamic-pituitary-adrenal; ICS: Intermittent cold stress; IL: Interleukin; NSAID: Non-steroidal anti-inflammatory drug; NT: Neurotrophin; PGE2: Prostaglandin E2; PKC: Protein kinase C; RVM: Rostrovventral medial medulla.

Competing interests

The authors declare that they have no competing interests.

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