Chapter 2

Animal Models of Inflammatory Pain

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Abstract

Animal models of inflammatory pain have been widely used to study the mechanisms of tissue injury-induced persistent pain. A variety of inflammatory agents or irritants, including complete Freund's adjuvant, carrageenan, zymosan, mustard oil, formalin, capsaicin, bee venom, acidic saline, lipopolysaccharide, inflammatory cytokines, and sodium urate crystals, have been used to produce tissue injury and hyperalgesia in such structures as cutaneous/subcutaneous tissues, joints, and muscles. Additionally, models of pain hypersensitivity have also been established with injuries produced by burning, freezing, and ultra irradiation. Although these models do not simulate every aspect of chronic pain, they do model key features of human inflammatory pain. Studies in animals give insight into certain aspects of human pain conditions and lead to improved pain management for patients.

1. Introduction

Pain perception is more complex in humans than in animals since human pain perception encompasses psychosocial, cultural, developmental, and environmental variables. However, human and animal pain perceptions show parallels, and animal models partially mimic the persistent pain encountered in the clinic. In the last two decades animal models of inflammatory pain have been widely used to study the mechanisms of tissue injury-induced persistent pain. Although none of the existing models can simulate all symptoms of inflammatory pain, studies in animals give insight into certain aspects of human pain conditions and lead to better pain management for patients. In the following paragraphs, commonly used inflammatory pain animal models will be summarized. Interested readers may consult more comprehensive reviews for further details (1, 2).

2. Inflammatory Models of Persistent Pain

Animal models of tissue injury and inflammatory hyperalgesia can be induced by a number of inflammatory agents in a variety of structures, including cutaneous and subcutaneous, joint, and muscle tissues.

2.1. Cutaneous and Subcutaneous Models of Inflammatory Pain

2.1.1. CFA Model

A *Mycobacterium butyricum* oil suspension was initially used to inoculate the tail base of the rat to induce adjuvant arthritis and persistent pain (3). Since polyarthritis develops after the inoculation along with a state of generalized illness, most pain researchers have discontinued the use of this model. However, the injection of complete Freund's adjuvant (CFA, composed of inactivated and dried *Mycobacterium* and adjuvant) into the footpad produces localized inflammation and persistent pain (4, 5). After a CFA injection into the footpad, cutaneous inflammation appears in minutes to hours and peaks within 5–8 h.

CFA produces dose-dependent inflammatory responses, and 30–200 μg of *Mycobacterium butyricum* suspended in oil/saline (1:1) yield significant edema and thermal hyperalgesia in the injected hind paw (6) (Fig. 1). The edema peaks around 24 h after the injection. The hyperalgesia and allodynia peak around 5 h after injection and persist for approximately 1–2 weeks (7).

CFA-induced hyperalgesia and allodynia in rats are consistent with those seen in humans receiving inadvert injections of
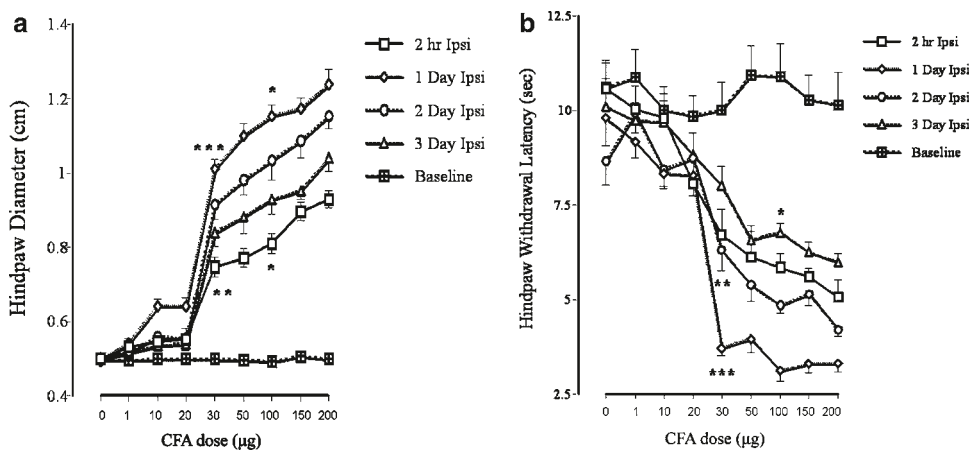

Fig. 1. Inflammation and hyperalgesia produced by intraplantar injection of complete Freund's adjuvant in rats. (**a**) Edema of the rat hind paw after injection of different doses of CFA, determined by measuring the dorsal-ventral thickness of the injected hindpaw. *$P < 0.05$ compared to 200 μg injected rats; **$P < 0.01$ compared to 20 μg injected rats; ***$P < 0.001$ compared to 20 μg injected rats. (**b**) Changes in hind paw withdrawal latency to a noxious thermal stimulus at different time points (2 h to 3 days) after injection of different doses of CFA into the hindpaw. *$P < 0.05$ compared to 200 μg injected rats; **$P < 0.01$ compared to 20 μg injected rats; ***$P < 0.001$ compared to 20 μg injected rats [reproduced with permission from Chinese Journal of Neuroanatomy (1999, 15:19–26), Chinese Society of Anatomical Science].

CFA (8). The physiological and biochemical effects of CFA are limited to the affected limb (5) and there are no signs of immune response or systemic disease. It has been shown that rats with CFA-induced inflammation exhibit minimal reductions in weight and show normal grooming behavior (5). Exploratory motor behavior is normal, and no significant alterations occur in an open field locomotion test (5).

Studies with three strains of rats, Lewis (LEW), Fischer 344 (FIS) and Sprague–Dawley (SD), demonstrate that, according to the difference scores computed by subtracting paw withdrawal latency (PWL) of the contralateral paw from that of the injected paw, F344 rats show significantly greater thermal hyperalgesia than do SD and LEW rats, both of which exhibit similar but relatively less intense hyperalgesia (9).

2.1.2. Carrageenan Model

An intraplantar injection of carrageenan is also widely used to produce a model of localized inflammatory pain. When 0.5 mg of carrageenan is injected, edema develops, mainly in two phases: the first 30 min after the injection, the second beginning at the end of the first hour and lasting until the third hour after injection. The edema peaks 3–5 h after injection (10, 11). When 6 mg of carrageenan is injected, edema peaks on day 3 (5) and thermal hyperalgesia peaks around 4 h after injection and lasts for at least 96 h (5). Studies with FIS, LEW, and SD rats demonstrate that LEW rats showed the least, and FIS rats the greatest, thermal hyperalgesia after intraplantar administration of 3.5 mg of carrageenan (12).

CFA and carrageenan are also injected into the facial area to study orofacial pain (13–15). A CFA injection into the perioral (PO) skin results in orofacial thermal hyperalgesia and mechanical allodynia that peak between 4 and 24 h and persist for at least 2 weeks (15). Facial carrageenan injection in mice causes increased responses to facial stimulation with a von Frey hair (1 g force) 8 h, 1 day, and 3 days after injection (13).

2.1.3. Mustard Oil and Zymosan Models

Other inflammatory agents such as mustard oil, a small fiber irritant, and zymosan, a glucan from the cell walls of yeast, have been used to produce behavioral hyperalgesia. Mustard oil elicits inflammatory pain by activating transient receptor potential cation channel, subfamily A, member 1 (TRPA1), an excitatory ion channel of primary afferent nociceptors (16). Topical application of mustard oil to the ear induces dose-dependent increases in plasma extravasation and ear thickness, which peak approximately 30 min after application (17). Application of mustard oil to rat paw skin induces plasma protein extravasation and slight edema, a 7–8% increase in paw volume (18). Topical application of mustard oil (20 μl, 100%) to the lateral surface of the left hind leg induces immediate agitation with frequent biting and

vocalizations. This response lasts approximately 5–7 min. Mustard oil also significantly facilitates a tail-flick reflex that appears 5 min after application and lasts up to 60 min, peaking 20 min after application (19).

Intraplantar injection of zymosan (0.31–6.25 mg) produces persistent dose- and time-dependent mechanical and thermal hyperalgesia. Edema is greatest at dosages ≥2.5 mg and peaks 30-min postinjection irrespective of dosage. Mechanical hyperalgesia appears at dosages ≥1.25 mg and reaches its maximum 4 h after application at a dosage of 5 mg. Thermal hyperalgesia is biphasic and dose-dependent. An early-phase peak occurs at 30 min at dosages ≥2.5 mg; this is not apparent at lower dosages. A late-phase peak occurs at 4 h at dosages of ≥0.0625 mg. Higher dosages (5 and 6.25 mg) also cause spontaneous pain, sometimes characterized by occasional flicking of the hind paw but more commonly by elevation of the paw for extended periods of time (20).

2.1.4. Formalin Model   The formalin test is a popular model for studying pain mechanisms under prolonged nociception. Formalin is injected beneath the footpad of a rat, mouse, or cat and produces two phases of nocifensive behavior, characterized by licking and flinching of the paw, that are separated by a short period of quiescence (21, 22). The first or acute phase occurs typically in the first 5 min; the second starts from 15 min and lasts about 40–60 min after injection. It is generally agreed that the first phase is due to the direct activation of both low-threshold mechanoreceptive and nociceptive primary afferent fibers (23). There has been disagreement about the underlying mechanisms of the second phase. Early studies suggested that the second phase resulted from an increase in the excitability of dorsal horn neurons. More recently, it has been demonstrated that ongoing activity of primary afferent fibers is necessary for the development of the second phase (23–26). In regard to the period of quiescence, some evidence supports the idea of an absence of activity, other evidence implicates an active inhibitory mechanism (27).

Formalin-induced pain is measured by combining scores of favoring, lifting, licking, and flinching/shaking of the injured paw. Power analysis indicates that using a moderate dosage (1.5%, 0.05 ml) of formalin and a combined pain score gives the greatest power to detect pain differences (22). Further, combining the formalin model with the place-conditional paradigm demonstrates that, when compared with a distinct environmental context, a hind paw injection of formalin induces conditioned place avoidance, which reflects a negative affective state (28).

To study orofacial pain, formalin is subcutaneously injected into the rat upper lip or lateral face and generates similar biphasic behavioral responses (face rubbing), an early and short-lasting first phase followed, after a quiescent period, by a second prolonged (tonic)

phase. The orofacial formalin test can be used to produce and quantify nociception in the trigeminal region of the rat (29–31). Study with different formalin concentrations indicates that the second phase response to formalin only occurs with dosages of 1.5% and higher (31).

2.1.5. Bee Venom Model

A subcutaneous injection of bee venom (0.2-mg lyophilized whole venom in 0.1-ml saline) into the hind paw produces persistent nociceptive responses (flinching and lifting/licking the injected paw) for 1–2 h, followed by a 72–96 h period of mechanical allodynia and thermal hyperalgesia accompanied by edema and redness of the injected paw. It also produces thermal hyperalgesia, but not mechanical allodynia, on the contralateral hind paw although with less amplitude than that of the injected paw (32).

2.1.6. Capsaicin Model

Capsaicin, the pungent component of cayenne pepper that activates transient receptor potential vanilloid type 1 (TRPV1), a heat-sensitive cation channel on nociceptor terminals, has been used in humans and animals to study neurogenic inflammation and hyperalgesia. Intradermal injection of capsaicin results in flare reaction, allodynia, and hyperalgesia, the areas of which extend beyond the injection site. Visual observation of flare response reveals that the area of visual flare is significantly smaller than the area of hyperalgesia to stroking stimuli and that the latter is significantly smaller than that for punctate stimuli. The heat hyperalgesia (thermode maintained at 38°C) area is the smallest (33, 34). Thermographic detection of the flare response shows that the thermographic area is larger than the area of visual flare and coincides with the area of mechanical (nylon monofilament, 1.02-mm diameter exerting a bending force of 2.02 N) and heat hyperalgesia (from a 1-cm² Peltier thermode maintained at 47°C) (35). Regarding the temporal pattern of flare, visual flare reaches its maximum within 3–5 min (33). Laser-Doppler flowmetry also shows that blood flow reaches maximum 5 min after the injection and then decreases (34). A thermographic device shows that the flare response starts as early as a few seconds after the capsaicin injection (35). The area of hyperalgesia to stroking stimuli appears immediately after injection, peaks within 15 min and then gradually decreases over 1–6 h. The area of hyperalgesia to punctate stimulation is immediately present after injection, grows to a maximum within 15–30 min, decreases gradually, and disappears at about 21 h. The area of the heat hyperalgesia reaches maximum 30 min after injection, gradually decreases, and disappears about 1.5 h after injection (33). Capsaicin (0.1, 1, 10, and 100 μg) produces dose-dependent increases in spontaneous pain, area and intensity of mechanical allodynia, area and intensity of pinprick hyperalgesia, and flare area (36).

It should be noted that capsaicin may produce differential responses in different areas. For instance, peak pain intensity and duration are greater in the forehead than in the forearm, while areas of visible flare and pinprick hyperalgesia are significantly larger in the forearm than in the forehead (37).

This neurogenic model of inflammation has been used in monkeys to study changes in nociceptor activity and changes in the responses of spinal dorsal horn neurons (33, 38). It has recently been adapted for behavioral studies in rats (39). Intraplantar injection of capsaicin evokes nocifensive behavior characterized by lifting and guarding of the injected paw that lasts for about 3 min. Capsaicin dose-dependently produces thermal and mechanical hyperalgesia. Thermal hyperalgesia to heat lasts up to 45 min, whereas mechanical hyperalgesia persists up to 4 h.

To study trigeminal pain, subcutaneous injection of different dosages of capsaicin into the vibrissa pad produces an immediate rubbing–scratching of the injected area. This behavior is performed with the ipsilateral forepaw, often accompanied by the contralateral forepaw. The rubbing–scratching response reaches its maximum during the 12- to 18-min interval and subsides about 42 min after capsaicin injection. Morphine dose-dependently reduces the capsaicin-induced rubbing–scratching (40, 41).

Further, capsaicin-sensitive primary afferents play different roles in chemical irritant-induced spontaneous nociception, hyperalgesia, and inflammatory responses (42). Local pretreatment with capsaicin significantly inhibits the two phases of formalin-induced persistent spontaneous nociception, while it only inhibits the late phase (tonic nociception; 11–60 min), not the early phase (acute nociception; 0–10 min), of spontaneous nociception in the bee venom test. Although capsaicin pretreatment prevents thermal hyperalgesia in the bee venom, carrageenan, and CFA models, it only prevents mechanical allodynia in the bee venom and carrageenan models, not the CFA model. Regarding inflammatory response, it significantly inhibits bee venom-elicited paw edema but not carrageenan-, CFA-, or formalin-elicited paw edema.

Rank order of the duration of the inflammatory hyperalgesia produced by these agents, is, from longest to shortest, CFA > bee venom > carrageenan > zymosan > formalin > mustard oil. See Table 1 for a comparison of onsets and durations.

2.2. Models of Joint Inflammation and Hyperalgesia

2.2.1. CFA-Induced Joint Inflammation

Adjuvant arthritis is induced in rats for a laboratory animal model of chronic pain that mimics that of human rheumatic disease. A CFA injection into the base of the rat's tail causes polyarthritis (43). Hypersensitivity of multiple joints occurs after 10 days and lasts up to 3 weeks. The paws and tails of arthritic rats show lower thresholds in response to noxious pressure (hyperalgesia), higher thresholds in response to noxious heat (hypoalgesia), and no change in response to noxious electrical stimulation (44).

Table 1
Comparison of cutaneous/subcutaneous inflammatory pain models[a]

Chemical	Hyperalgesia	Allodynia	Time of onset	Duration
CFA	Yes	Yes	2–6 h	1–2 weeks
Carrageenan	Yes	Yes	1 h	24 h
Mustard oil	Yes	Yes	5 min	<1 h
Zymosan	Yes	Yes	30 min	24 h
Formalin phase I	N/A[b]	N/A	<1 min	5–10 min
Formalin phase II	N/A	N/A	10 min	1 h
Bee venom	Yes	Yes	1 min	96 h
Capsaicin	Yes	Yes	1 min	<1 h

[a]Modified and reprinted with permission from the ILAR Journal, 40(3),1999, Institute for Laboratory Animal Research, The National Academics, 500 Fifth Street NW, Washington, DC 20001 (http://www.national-academies. org/Ilar)
[b]Not applicable

Pain is also inferred from scratching behaviors, reduced motor activity, weight loss, vocalization when the affected limbs are pinched, and a reduction in these behaviors following the administration of opioids. It should be noted that this is a systemic disease that includes skin lesions, destruction of bone and cartilage, impairment of liver function, and lymphadenopathy, which leads to ethical concerns (45). Moreover, the systemic lesions make it difficult to differentiate pain behavior from generalized malaise and debilitation. The likely presence of central nervous system changes associated with the alterations in immune function also call into question the use of this model as a correlation of pain behavior to neural activity and neurochemical alterations. Polyarthritis is also induced by type II collagen emulsified in Freund's incomplete adjuvant and injected into the base of the rat's tail. Tail-flick latency significantly decreases 1 week after inoculation, peaks at 3 weeks, and lasts for at least 5 weeks (46). Like CFA-induced polyarthritis, collagen-induced polyarthritis raises ethical concerns.

An alternative to the polyarthritic rat for prolonged studies, intra-knee joint injection of 250-mg suspension of heat-killed *Mycobacterium butyricum* in peanut oil and saline (1:1), causes ipsilateral thermal hyperalgesia of the hind paw 1 day after injection, peaks on day 3, and remains at that level until day 14. By the 21st day, experimental animals recover from the hyperalgesia (47). Knee joint withdrawal threshold measurement, in which a gradually increasing squeeze is applied across the joint, shows that the average ipsilateral limb withdrawal threshold (LWT) significantly decreases by 57%, from 790 ± 39 to 316 ± 45 g, 1 day

after the CFA injection. This decrease lasts for a full 28 days, the period studied. An incapacity test shows that the ratio of weight distribution between ipsilateral and contralateral limbs significantly decreases from 0.96 ± 0.03 to 0.29 ± 0.05. This significant decrease lasts up to day 28 (48).

Injection (0.05 ml) of 300 μg *Mycobacterium butyricum* into the tibio-tarsal joint also produces monoarthritis. As revealed by clinical observations and X-ray examinations, the arthritis is limited anatomically, pronounced, prolonged, and stable from weeks 2 through 6 postinjection. The affected limb shows a marked increase in sensitivity to paw pressure. Animals gain weight, remain active, and evince little systemic disturbance in contrast to polyarthritic rats (49).

CFA also produces significant thermal hyperalgesia and mechanical allodynia following its injection into the temporomandibular joint. Thermal hyperalgesia develops at 5 h, peaks at 24 h, and lasts 2 weeks. Mechanical allodynia starts at 2 h, peaks between 4 and 24 h and persists for at least 2 weeks after the injection (15).

2.2.2. Kaolin and Carrageenan-Induced Joint Inflammation

Another arthritis animal model is induced by injecting kaolin and carrageenan (3 mg/3 mg) into the knee joint. In rats, decrease in PWL occurs ipsilaterally to the inflamed knee as early as 4 h after an injection of the two agents and lasts about 24 h. The circumference of the ipsilateral knee joint is significantly larger than baseline between 4 and 24 h. The rats also show spontaneous pain, indicated by decrease of weight bearing by the injected limb (50, 51). Recently, the elevated plus maze test has demonstrated that arthritic rats show amygdala-involved anxiety-like behavior, evidenced by a decreased preference for the open arms (52). Intra-articular injection of carrageenan and kaolin in cats causes guarding of the leg and avoidance of movement or weight bearing. These symptoms begin ~2 h after the injection, are fully developed after 4 h, and last at least 15 h.

Recording of saphenous nerve filament activity consistently demonstrates that almost all filament units of inflamed joints have low thresholds to passive movement of the knee joint compared to units of normal joints. The number of receptive fields per unit is significantly greater than that seen in normal joints (53). In rats, cats, and monkeys, somatosensory neurons of the spinal cord become hyperexcitable to mechanical stimuli during the development of experimental arthritis (54). Changes in joint receptors and spinal dorsal horn neuronal activity begin as soon as 1–2 h following injection and build for several hours. Noticeably, the magnitude of hyperalgesia in this model is relatively low compared to that of the hind paw inflammation model. It should be kept in mind that the test site, the paw, in the joint inflammation model is remote from the injury site. What is measured by PWL in the knee joint inflammation model is, likely, only secondary hyperalgesia.

2.2.3. Carrageenan-
Induced Joint
Inflammation

Compared to the short-lasting effects of the kaolin and carrageenan combination, carrageenan alone produces a long-lasting effect. An intra-articular injection of 3 mg of carrageenan significantly decreases ipsilateral and contralateral PWL to heat; the decrease occurs at 4 h and lasts 6 weeks. Carrageenan at 0.3 and 1 mg produces only ipsilateral effects that are shorter-lasting: 24 h for 0.3 mg and up to 3 weeks for 1 mg. Intra-articular injection of 3 mg of carrageenan also produces significant decrease of the mechanical withdrawal threshold ipsilaterally (between 3 and 7 weeks) and contralaterally (between 3 and 6 weeks). Carrageenan at 1 mg induces a significant ipsilateral decrease between 4 h and 1 week, but 0.3 mg has no effect (55).

2.2.4. Formalin-Induced
Joint Inflammation

Formalin (0.5, 3, and 5%) injected into the knee joint of rats induces dose-dependent nocifensive responses. The nociception consists of two phases (from 0 to 5 min and from 10 to 60 min) of intense guarding behavior on the affected limb with an intervening period of quiescence (from 5 to 10 min). Morphine (4 mg/kg, subcutaneously) pretreatment reduces the guarding behavior in both nocifensive phases (56). A formalin (0.5, 2.5, and 5%) injection into the temporomandibular joint (TMJ) region induces a dose-dependent phase of orofacial rubbing and one of flinching, alternately displayed. The rubbing responses start earlier, peak 18 min postinjection, and then decrease; the flinching responses start later, peak 27 min postinjection, and last up to 36 min. A significant correlation exists between formalin concentration and rubbing and flinching responses. The magnitude of these responses reaches its maximum at a concentration of 2.5% (57).

2.2.5. Joint Inflammation
Induced by Other Irritants

Other models of arthritis have been developed using sodium urate crystals, which are injected into the ankle joint of a rat or cat (45, 58). The arthritis is fully developed within 24 h. These animals tend to place less weight on the treated hind limb and exhibit guarding movements of the limb. In the rat, touch, pressure and thermal stimuli applied to the affected paw result in a decrease in responsiveness, presumably due to pain associated with the movement. There are no signs of systemic disease in the urate arthritis model other than the joint pathology secondary to tissue edema and the infiltration of polymorphonuclear leukocytes (45).

Capsaicin (0.2%, 50 μl) injected into the lateral aspect of the left ankle joint results in a decreased mechanical withdrawal threshold 2 h after injection; this is maintained through a 4-h period (59). Capsaicin-sensitive primary afferents play different roles in a variety of joint inflammation models, as they do in cutaneous/subcutaneous pain models. Pretreating the joint with 1% capsaicin (about 1 week before injection) significantly reduces the inflammatory response to carrageenan and urate but not to formalin (60). Onsets and pain durations produced by joint inflammation are compared in Table 2.

Table 2
Comparison of joint inflammatory pain models

Chemical	Hyperalgesia	Allodynia	Time of onset	Duration
CFA	Yes	Yes	1 day	3–4 weeks
Carrageenan	Yes	Yes	4 h	6 weeks
Formalin phase I	N/A[a]	N/A	<1 min	5 min
Formalin phase II	N/A	N/A	10 min	1 h
Capsaicin	N/A	Yes	2 h	4 h

[a]Not applicable

2.3. Models of Muscle Inflammation and Hyperalgesia

2.3.1. Acidic Saline-Induced Muscle Inflammation and Hyperalgesia

The bulk of available knowledge about pain mechanisms is derived from studies on cutaneous pain. However, the existing subjective differences between muscle and skin pain (e.g., muscle pain is poorly localized and shows referral) suggest that muscle pain has distinct characteristics. Models have been developed to examine mechanisms underlying the development and maintenance of chronic muscle pain.

A decrease in tissue pH has been observed in response to inflammation, hematomas, and isometric exercise. Decreasing pH increases activity of nociceptors and produces a painful response in humans (61). Using an in vitro nerve-skin preparation, continuous infusion of low pH (5.2–6.9) saline increases discharges of C-polymodal primary afferent fibers without adaptation (62). Repeated injection of low pH saline into the gastrocnemius muscle of rats produces long-lasting, widespread mechanical hyperalgesia without motor deficits or significant tissue damage.

Following the first unilateral intra-gastrocnemius muscle injection of pH 4.0, 5.0, or 6.0 saline, the mechanical withdrawal threshold of the ipsilateral paw dose-dependently decreases 4 h, and returns to baseline 24 h, after injection. After a second unilateral injection of low pH saline on day 5, the mechanical withdrawal threshold dose-dependently decreases in both ipsilateral and contralateral hind paws. These bilateral decreases are greatest for pH 4.0 saline, persisting for 4 weeks after the second injection. Inter-injection intervals of 2 and 5 days (pH 4.0 saline) produce equivalent and significant bilateral decreases in mechanical withdrawal threshold. A 10-day inter-injection interval does not produce persistent mechanical hyperalgesia. This suggests that there is a critical window in which re-injury to muscle tissue results in exaggerated, more persistent hyperalgesia. Intra-gastrocnemius muscle lidocaine 24 h after the second injection of pH 4.0 saline increases the ipsilateral mechanical withdrawal threshold during the first 10–15 min after injection. However, lidocaine has no significant effect on the decreased contralateral withdrawal threshold.

Withdrawal latencies to radiant heat average approximately 10 s at baseline and are no different than controls after either the first or second injection of low pH saline. After both the first and the second injection of low pH saline, rats show no limb guarding, have equal weight bearing and normal gait patterns, and their ability to perform the treadmill test is unchanged (63). Interestingly, in sharp contrast to most persistent pain models intramuscular acidic saline-induced allodynia does not involve spinal glial activation or inflammatory cytokine interleukin-1 (64).

2.3.2. Carrageenan-Induced Muscle Inflammation and Hyperalgesia

Intramuscular application of carrageenan sensitizes group III and IV muscle afferents, including nociceptors, lowering their thresholds to mechanical activation and increasing their background activity. Furthermore, carrageenan induces local inflammation when injected into the muscle, as evidenced by the accumulation of leukocytes that begins 2 h postinjection and continues for the next 8 h (65).

Injection of carrageenan (0.5–6 mg/triceps) into the bilateral triceps muscles produces dose-dependent reduction in forelimb grip force that peaks 24 h, and returns to the control level 48 h, postinjection. Capsaicin (50 mg/kg i.p.) admi nistration to rats on the second day of life reduces carrageenan-evoked hyperalgesia by about 45%, indicating that the muscle hyperalgesia induced by carrageenan is mediated, in part, by capsaicin-sensitive afferent fibers (66).

Unilateral intra-gastrocnemius muscle injection of carrageenan also dose-dependently produces thermal and mechanical hyperalgesia. Carrageenan at 3 mg significantly decreases ipsilateral PWL to heat that occurs within 4 h and lasts 8 weeks. Contralateral PWL also decreases by the end of the first week and last 8 weeks. Carrageenan at 0.3 and 1 mg produces no significant effect on PWL to noxious thermal stimulus (55). However, a 3-mg intramuscle injection of carrageenan significantly decreases the ipsilateral mechanical withdrawal threshold between 4 h and 6 weeks and the contralateral threshold between weeks 3 and 6. The ipsilateral mechanical withdrawal threshold significantly decreases between 4 and 8 h after a 1-mg intramuscle injection of carrageenan. Carrageenan at a dosage of 0.3 mg produces no changes in mechanical sensitivity (55). It seems that mechanical sensation is more sensitive to carrageenan than is thermal sensation.

2.3.3. Cytokine-Induced Muscle Inflammation and Hyperalgesia

An intra-gastrocnemius injection of tumor necrosis factor-alpha (TNF) significantly decreases mechanical withdrawal thresholds to muscle pressure in rats when measured with an algesimeter that exerts pressure on the gastrocnemius muscle. It also decreases forelimb grip strength as measured with a digital grip force meter. The hyperalgesia lasts at least 60 min (67). Similarly, an intra-gastrocnemius injection of another pro-inflammatory cytokine

interleukin-6 significantly decreases the mechanical threshold, which returns to normal levels 5 days after the injection (68).

2.3.4. Mustard Oil, Formalin, and Hypertonic Saline

Mustard oil (30 µl, 20%), formalin (50 µl, 3%), or hypertonic saline (100 µl, 5%) injected in the mid-region of the masseter muscle elicits significant hind paw shaking behavior compared to an injection of vehicle. The peak and overall magnitude of this behavior present in a dose-dependent manner (mustard oil 1–20%) and is dose-dependently attenuated by the systemic administration of morphine sulfate (69). CFA injected into the bilateral masseter muscles of the rat significantly decreases the bite force on days 1, 2, and 3. The bite force gradually increases and then returns to baseline by day 14 (70). Onsets and durations of pain produced by muscle inflammation are compared in Table 3.

2.4. Other Inflammatory Pain Models

2.4.1. Burn Injury Model

Burn injury-induced pain model has been developed in rats. A mild focal thermal injury (52°C/30–45 s) to the rat heel induces primary thermal and mechanical hyperalgesia that lasts for 2 h and secondary mechanical hyperesthesia and tactile allodynia that last 3 and 2 h, respectively (71). After a third-degree burn injury induced by immersing the dorsal part of the right hind paw into a hot water bath (85°C/12 s), the rats display thermal hyperalgesia and mechanical allodynia that are evident by day 1, peak around days 5–7, and persist for at least 2 weeks (72). The dose–response curve of morphine shifts to the right in burn-injured rats as compared to sham rats on postinjury day 14 (72). This is in contrast to carrageenan-induced inflammatory pain, in which the dose–response curves for intrathecal mu- and delta-opioid receptor agonists shift to the left for inflamed hind paws compared to contralateral, uninflamed paws (73).

A burn injury human model has been reported. The injury is produced on the medial part of the nondominant crus, and pain thresholds decrease and pain responses increase in reaction to both thermal and mechanical stimuli within the burn area according to a visual analog scale (0–100). The burns also induce

Table 3
Comparison of muscle inflammatory pain models

Chemical	Hyperalgesia	Allodynia	Time of onset	Duration
Acidic saline	No	Yes	4 h	24 h (one inj.) or 4 weeks (two inj.)
Carrageenan	Yes	Yes	4 h	8 weeks (hyperalgesia) 6 weeks (allodynia)
Interleukin-6	N/A	Yes	30 min	3 h to 5 days

secondary mechanical and thermal hyperalgesia and increased pain response to mechanical and heat stimuli in the area of secondary hyperalgesia (74).

2.4.2. Freeze Injury Model

Recently, a freeze injury-induced pain model has been developed in humans. The end (1.8 cm²) of a cylindrical copper bar previously cooled to –28°C is applied on the anterior glabrous part of the forearm to induce a first-degree burn injury. Several hours after the injury, sharply delimited erythema accompanied by localized hyperalgesia is observed. Tissue injury leads to primary mechanical hyperalgesia, limited to the area of injury, and to secondary mechanical hyperalgesia in the undamaged skin surrounding the injury. The hyperalgesia lasts at least 70 h and can be significantly relieved by systemic or topical ibuprofen. This is a useful tool for evaluating the efficacy and detecting the potential sites of action of analgesic agents such as nonsteroidal anti-inflammatory drugs in healthy human subjects (75).

2.4.3. Ultraviolet Irradiation Model

Acute cutaneous over-exposure to ultraviolet radiation (UVR) has been used to construct inflammatory pain models in both rats and humans. UVB (280–320 nm) irradiation of the hairy skin of rat hind paws dose-dependently produces erythema, thermal hyperalgesia, and mechanical allodynia (76). Erythema occurs on day 1 postirradiation, peaks on day 2, and returns to normal between days 4 and 7 depending on the UV dosage. Thermal hyperalgesia and mechanical allodynia appear on day 1, peak on day 2, and return to normal between days 7 and 14 for thermal sensation and days 4 and 14 for mechanical sensation, depending on the UV dosages. Systemic morphine produces dose-dependent and naloxone-sensitive reversal of the sensory changes.

In humans, UV irradiation also causes erythema and thermal hyperalgesia and mechanical allodynia (77). Solar-simulated radiation (SSR) at three minimal erythema dosages (MED) induces significant erythema 3 h postirradiation that peaks in 24 h and lasts at least 72 h. SSR dose-dependently produces significant thermal hyperalgesia 24 h postirradiation that lasts at least 72 h, and mechanical hyperalgesia in 6 h that peaks in 48 h and lasts at least 72 h.

2.4.4. Toxin-Induced Pain Models

Snakebites are a public health problem in Central and South America. It has been reported that an intraplantar injection (5–20 μg/paw) of Lys49 or Asp49 phospholipases A2 from *Bothrops asper* snake venom causes mechanical hyperalgesia, measured with the paw pressure test, which peaks in 1 h and normalizes 24 h after the injection (78).

In the aforementioned inflammatory pain models, the peripheral somatic nociceptors are stimulated and the nociceptive inputs

are directly transmitted to the spinal cord or to trigeminal sensory nuclei. In contrast, intraperitoneally administered lipopolysaccharide (LPS; bacterial endotoxin) does not result in direct nociceptive input to the spinal cord dorsal horn but produces a long-lasting facilitation of the nociceptive tail-flick reflex (79). It has been shown that LPS-induced hyperalgesia is blocked by hepatic vagotomy and descending funiculus lesions but not by transection of the splanchnic nerve, the primary afferent for visceral pain (80). LPS-induced hyperalgesia lasts at least 60 min.

3. Conclusion

A variety of inflammatory pain animal models have been established by mimicking the injury of different tissues of skin, muscle, and joint. Although these models do not simulate every aspect of chronic pain symptoms, they do imitate some key features of human inflammatory pain. Through studies with a variety of persistent pain models, enormous progress is being made in the discovery of the cellular and molecular mechanisms responsible for the pathogenesis of pain.

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