CHAPTER 8:

DEVELOPMENT AND VALIDATION OF MUSCULAR PAIN: POSSIBLE IMPLICATIONS IN MEDIATING HYPERALGESIA
8.1. UNILATERAL INTRAMUSCULAR INJECTION OF LIPOPOLYSACCHARIDE PRODUCES BILATERAL HYPERALGESIA

8.1.1. INTRODUCTION
The strategies for treatment and prevention of musculoskeletal pain syndrome are not optimal. Muscle pain is cramp like, diffuse, and the pain is referred to distance somatic structures and causing the superficial and deep sensitivity in the painful areas. The manifestations of muscle pain are different from cutaneous pain, which is superficial, is localized at and around the injury (Arendt-Nielsen et al., 1996; Graven-Nielsen and Mense 2001). Chronic pain conditions, such as fibromyalgia and musculoskeletal pain, are characterized by wide spread muscle pain and joint tenderness (Wolfe et al., 1990; Mense et al., 1993). Further, it is generally accepted that chronic fatigue syndrome is also associated with musculoskeletal pain. A recent report of enhanced temporal summation, also known as wind up, in fibromyalgia patients and experimental animals, consistent with central sensitization (Price et al., 2002; Bennett, 2005), suggest that the etiological development of chronic musculoskeletal pain in humans may share a number of common underlying mechanisms with other chronic pain conditions including those of neuropathic origin (Zimmerman 1991). It is generally believed that both peripheral and central mechanism(s) have been involved in the pathogenesis of muscular pain (Graven-Nielson and Mense 2001; Bennett, 2005). Similar to neuropathic pain in humans, chronic musculoskeletal pain remain somewhat refractory to treatment with currently available analgesics reinforcing the need to understand complex pathophysiological mechanisms involved in the development and maintenance of muscle pain.

In the past, studies have been hampered by the lack of animal models for muscle pain. More recently, models using intramuscular injection of acidic saline (Sluka et al., 2001), carrageenan (Radhakrishnan et al., 2003), or capsaicin (Sluka et al., 2002) have been established that results in long lasting hyperalgesia and allodynia. Acidic saline causes hyperalgesia particularly due to changes in pH around muscle nociceptors and altering the function of acid sensitive ion channels (Sluka et al., 2003), carrageenan produces non-immune-mediated inflammation (Nicklin and Miller, 1984) whereas capsaicin produces a neuronal activation (Soliman et al., 2005) that results in hyperexcitability and release of neurotransmitters and/or
neuromodulators. Accumulating data indicates that immune factors and neuron-immune interactions play an important role in facilitating nociception and mediating hypersensitivity (Meller et al., 1994; Watkins et al., 1997; Sweitzer et al., 2001). Therefore, these models may not be appropriate to study specific immune factors that are prevalent and neuroimmune interaction that might be contributed in the manifestation of hypersensitivity in fibromyalgia and chronic fatigue syndrome. A better understanding of the involved basic mechanisms and better models to assess muscle pain may provide new possibilities for designing rational therapies and for targeting the pharmacologic intervention optimally.

Lipopolysaccharide, also known as endotoxin, has been studied extensively with respect to its mechanical and thermal hyperalgesic effects in rats (Maier et al., 1994; Kanaan et al., 1996; Jain et al., 2001). It is now well established that peripheral or central administration of LPS reacts with various cell types to release cytokines IL-1, IL-6, and TNF-α in the periphery and CNS, which can in turn precipitate hyperalgesia (Maier et al., 1994; Reeve et al., 2000). A single high dose LPS decreases mechanical nociceptive thresholds in rats and mice, consistent with widespread muscle aches and pain reported in humans during endotoxemia (Hochstein et al., 1994; Lynn et al., 2003). As yet, there is no direct evidence that LPS induces muscular pain in animals and the potential of LPS in inducing muscular pain has not been evaluated so far.

Therefore, the present study examined whether intramuscular injection of LPS can induce behavioral indices of hypersensitivity such as mechanical hyperalgesia in the rat hindpaw.

8.1.2. MATERIALS AND METHODS

8.1.2.1. Experimental animals
As per 1.2.1. Male Wistar rats (bred in Central Animal House of Panjab University, Chandigarh) weighing 180-200 g at the start of the experiment were randomly divided into various groups consisting of 6 - 8 animals per group.

8.1.2.2. Induction and characterization of lipopolysaccharide-induced hypersensitivity
A single injection of LPS 10, 30 or 100 μg/0.1 ml/rat was administered intramuscularly in the left gastrocnemius muscle. Control animals received normal saline 0.1 ml/rat.
8.1.2.3. Behavioral assessment of nociception

Mechanical hyperalgesia (decreased threshold to mechanical stimuli) were evaluated in ipsilateral and contralateral paw of rats. The withdrawal threshold was evaluated using an analgesymeter (Ugo Basile, Italy) by applying noxious pressure to hind paw and expressed in grams as per 1.2.2.3b.

8.1.2.4. Assessment of motor function

Motor impairment following LPS administration was evaluated by placing/stepping reflexes according to the procedure described by Sluka et al. (2001). Motor impairment was examined after every one hour for 4 h. For placing reflex, rats had the dorsum of either hindpaw drawn across the edge of the table. This elicits a lifting of the paw onto the surface of the table that was scored as 2 = normal, 1 = delay of 1-2 sc, 0 = delay of more than 2 sec. Stepping reflexes were assessed subjectively, the gait abnormalities of each LPS injected animal was observed for 2 - 4 min, and scored as 2 = normal, 1 = limping, and 0 = paralysis of the hindlimb injected with LPS.

8.1.2.5. Materials and treatment schedule

Lipopolysaccharide from Salmonella typhimurium (Sigma, USA) was used in this study. LPS dissolved in normal saline to get a desired concentration of LPS in 0.1 ml. A single injection of LPS 10, 30 or 100 µg/0.1 ml/rat was administered intramuscularly in the left gastrocnemius muscle. All animals were acclimatized to laboratory environment for at least 2 h before testing. The ipsilateral and contralateral paw withdrawal responses to mechanical stimulation were measured on day 0 (before), 1, 2, 3, 4, 24, 48, 72 h, and 1 week after injecting LPS.

8.1.2.6. Statistical analysis

As per 3.1.2.5.

8.1.3. RESULTS

8.1.3.1. Induction and characterization of lipopolysaccharide-induced hypersensitivity

In the present series of experiments, the baseline paw withdrawal response in each test obtained on day 0 for each rat was relatively stable and showed no significant variation. The mean paw withdrawal threshold to pressure was 215.33 ± 16.58 g and 217.63 ± 18.67 g, respectively, on day 0 before injecting LPS. The paw withdrawal responses to mechanical stimulation in saline-injected rats remained unchanged from baseline values throughout the entire observation period. The ipsilateral and
contralateral paw withdrawal responses of rats injected with LPS (10 μg) were not significantly different from that of saline-injected rats (Fig. 8.1.1A and 1B). In contrast, administration of LPS (30 or 100 μg, i.m.) produced a marked decrease in ipsilateral paw withdrawal responses from one hour onwards and reached steady state responses between 4 and 48 h after LPS injection indicating the development of hyperalgesia in a dose- and time-dependent manner (Fig. 8.1.1A). However, the ipsilateral paw withdrawal responses in these animals started restoring to normal from 72 h onwards and reached to baseline levels 1 week after LPS administration. Contralaterally, there was similar and significant decrease in paw withdrawal responses to pressure in rats injected with LPS (30 or 100 μg), however, the paw withdrawal responses restoring to baseline levels normal from 72 h onwards after LPS injection (Fig. 8.1.1B).

8.1.3.2. Assessment of motor function

Following intramuscular administration of LPS, the animals were healthy, exhibited normal grooming and feeding behavior, and gained weight normally. The placing reflex was normal for all LPS-injected animals and there was no change in the ability to lift the paw onto the surface when hindpaw drawn across the edge of the table. Subjective assessment of stepping reflex in LPS-injected animals showed no limb guarding, equal weight bearing, and normal gait pattern.
8.1.4. DISCUSSION

In the present study, intramuscular administration of LPS decreased paw withdrawal threshold to mechanical stimulus, which is an indicative of hyperalgesic response. The important aspect in this study is the time course of hyperalgesia that was most evident and consistent in all the LPS-treated animals. Although there was no significant difference in the paw withdrawal threshold between saline and LPS (10 µg)-treated animals throughout the observation period, but there was a marked and significant potentiation of nociceptive response after LPS (30 or 100 µg) treatment in rats resulting in a state of hyperalgesia. Previous studies have shown increased sensitivity of dorsal horn neurons to mechanical stimulation that follows systemic, spinal or hind paw injections of LPS (Maier et al., 1994; Kanaan et al., 1996; Reeve et al., 2000). Indeed, direct spinal administration of LPS and cytokines produced an activation of dorsal horn neurons (Reeves et al., 2000; Svensson et al., 2005a). These
electrophysiological observations suggested that direct effects of LPS on the behavioral hyperalgesia. It is noteworthy that trauma, surgery, and infection, three conditions that increase circulating endotoxins, are anecdotally reported events in patients before the onset of fibromyalgia syndrome, a condition characterized by muscle and tactile hyperalgesia (Larson and Kovacs, 2001). Indeed, fibromyalgia frequently co-exists with conditions associated with endotoxemia supports that the present results of LPS-induced hypersensitivity to mechanical stimulation are particularly important in this condition and further this model may be useful in elucidating mechanisms involved in such conditions.

It has been well reported that LPS-induced hyperalgesia represents facilitated state of central sensitization. In the recent past, a number of studies attempted to unravel the mechanisms underlying in LPS-induced hyperalgesia (Anjaneyulu et al., 2003; Jain et al., 2001; Matsumoto et al., 1998; Safieh-Garabedian et al., 1997). LPS, when administered intraperitoneally does not cross blood-brain barrier, however, it activates non-neuronal cells and also stimulates the expression and release of various immunological factors, cytokines such as IL-1, IL-6, and TNF-α by activated monocytes and macrophages and proinflammatory mediators in the periphery and in the central nervous system (Minami et al., 1994; Kreutzberg, 1996; Weiseler-Frank et al., 2004). Further, it has also been reported that intramuscular administration of TNF-α induces muscle hyperalgesia in rats (Schaefers et al., 2003). These proinflammatory cytokines directly sensitize neurons and act indirectly in an autocrine or paracrine fashion to induce the synthesis and secretion of neurotransmitters that act on local neurons lead to hyperexcitable sensory states, which promote the development of hypersensitivity (DeLeo and Yezierski, 2001; Watkins et al., 2001). In addition, these cytokines activate microglial cells thereby increase the expression of iNOS, and COX-2 in the neuronal and non-neuronal elements, such as astrocytes and microglia, besides macrophages and fibroblasts following LPS administration (O’Neill et al., 1989; Matsumoto et al., 1998; Tonoji et al., 1999; Eriksson et al., 2000; Samad et al., 2001; Schuligoi et al., 2003). In the spinal cord, LPS activates the signaling pathways such as NF-κB, AP-1, CREB, MAPK cascade led to transcriptional activation of various enzyme expressions in astrocytes, microglia, endothelial cells, and leptomeningeal cells (Hwang, 1997; Samad et al., 2001; Koistinaho and Koistinaho, 2002; Shi and Gaestel, 2002). Previous studies have shown that the dramatic increase
in the levels of activated microglia in spinal cord in response to nerve injury (Sweitzer et al., 2001; Raghavendra et al., 2002; Ledeboer et al., 2005). It is well reported that the increased expression of COX-2 enhances in basal and evoked release of PGs, which sensitize peripheral nerve endings and facilitate central nociceptive processing in spinal cord results in exaggerated pain behavior (hyperalgesia). Thus, neuroimmune activation is the most likely explanation for these changes and consequent hypersensitivity following LPS administration.

The interesting finding of the present study was the presence of hypersensitivity contralateral to paws injected with LPS. Previous studies have reported the bilateral effects following unilateral injury and reviewed by many investigators (Koltzenberg et al., 1999; Lowrie, 1999; Chacur et al., 2001; Radhakrishnan et al., 2003). Recently, it has been shown that unilateral intramuscular injection of acidic saline or carrageenan produces bilateral hyperalgesia and further contralateral flexion reflex was not prevented by blockade of afferent input by unilateral dorsal rhizotomy or lidocaine pre-treatment (Sluka et al., 2001; Radhakrishnan et al., 2003). Further, intraplantar injection of bee venom into hind paw also produces bilateral hypersensitivity to thermal and mechanical stimuli in both ipsilateral and contralateral paw that was not abolished by ipsilateral sciatic nerve axotomy. However, contralateral paw responses were prevented by spinal administration of NMDA and non-NMDA receptor antagonists indicating that nociceptor mediators cause spinal excitability that leads to central sensitization (Chen et al., 2000; Skyba et al., 2002). Moreover, it is well reported that perineural administration of zymosan, yeast cell wall component, produces bilateral hyperalgesia, which is dependent on spinal cord glial activation and the release of proinflammatory cytokines (IL-1β, TNF-α, IL-6). Thus, it is plausible that contralateral spread of hypersensitivity likely depends on neuroplastic changes in the CNS (Milligan et al., 2003). Together, the data indicate that the immune activation might be a major contributor to hypersensitivity after intramuscular administration of LPS.

The results of the present study clearly demonstrate that intramuscular administration of LPS induces muscular pain that resembles fibromyalgia and chronic musculoskeletal pain.
8.2. POSSIBLE ROLE OF MICROGLIA IN LIPOPOLYSACCHARIDE-
INDUCED MUSCLE HYPERALGESIA

8.2.1. INTRODUCTION
Chronic pain conditions such as musculoskeletal pain and fibromyalgia are characterized by widespread muscle pain and joint tenderness (Wolfe et al., 1990; Bennett, 2005) and are the most frequent symptoms encountered in primary care providers (Gallagher et al., 2004). However, the mechanisms that participate in this pain are not completely understood so far. It has been identified that peripheral and central sensitizations are involved in the manifestations of musculoskeletal pain disorders. Further, it has become increasingly evident that muscle hyperalgesia, referred pain, referred hyperalgesia, and widespread hyperalgesia play an important role in chronic musculoskeletal pain. Recent findings of enhanced temporal summation, also known as wind up, in fibromyalgia patients, consistent with central sensitization (Price et al., 2002; Staud, 2002; Bennett, 2005), suggest that the etiological development of chronic musculoskeletal pain in humans may share a number of common underlying mechanisms with other chronic pain conditions including those of neuropathic origin (Zimmerman, 1991). However, there are no experimental studies directed to understand these mechanisms of musculoskeletal pain. Thus, a better understanding of the involved basic mechanisms, better animal models, and methods to assess muscle pain provide new therapeutic targets, possibilities for designing rational therapies and for targeting the pharmacologic intervention optimally.

The role of neurons and neurotransmitters in nociception has been well established, and the role of non-neuronal cells, such as glia, and their secretory products in the development of hyperalgesia has been studied recently. Both microglia, the intrinsic macrophages of the CNS, and astrocytes release a variety of proinflammatory cytokines (IL-1, IL-6, and TNF-α), which play a role in mediating or maintaining hypersensitivity following nerve injury and inflammation (Meller et al., 1994; DeLeo and Yezeirski, 2001; Fu et al., 2001; Watkins et al., 2001). It has also been found that LPS activates glial cells in both in vitro and in vivo (Ogata et al., 2003; Schuligoi et al., 2003). Recent studies have shown that spinal microglial cells are activated following nerve injury, perineural injection of zymosan and human immunodeficiency virus-1 (HIV-1) envelope glycoprotein gp120, and induce...
hypersensitivity (Milligan et al., 2001; 2003; Ledeboer et al., 2005). Moreover, in rat models of neuropathic pain and formalin-induced hyperalgesia, modulators of glial activation were shown to decrease allodynia (Fu et al., 2001; Raghavendra et al., 2003a; Wu et al., 2004). However, less is known about the contribution of glial cells in the manifestation of hypersensitivity in muscular pain.

In the past, studies have been hampered by the lack of animal models for muscle pain. In the last five years, a number of animal models of neuropathic pain have been developed and characterized (Sluka et al., 2001, 2003; Radhakrishnan et al., 2003). There are a number of shortcomings with these animal models, which provide important clues in understanding the underlying pathophysiology of muscular pain in humans. The second part of this chapter discusses the induction and characterization of lipopolysaccharide-induced hyperalgesia. Accumulating data indicates that immune factors and neuron-immune interactions play an important role in facilitating of nociception and mediating LPS induced hypersensitivity (Meller et al., 1994; Kanaan et al., 1996; Reeve et al., 2000; Jain et al., 2002). Therefore, this model may be appropriate to study specific immune factors that are prevalent and neuroimmune interaction that might be contributed in the manifestation of hypersensitivity in fibromyalgia, chronic fatigue syndrome and chronic musculoskeletal pain.

Thus, the present study was carried out to establish and characterize intramuscular LPS-induced mechanical hyperalgesia in the rat hindpaw. To date, there are no studies that examined the role of non-neuronal cells, particularly microglial cells, on persistent and long lasting muscle hyperalgesia. To test this hypothesis, the present study evaluated the effects of minocycline, an inhibitor of microglial activation, on LPS-induced muscle hyperalgesia. In addition, post-treatment effects of minocycline were also evaluated to delineate the role of microglial activation on the development and maintenance of LPS-induced muscle hyperalgesia.

8.2.2. MATERIALS AND METHODS
8.2.2.1. Experimental animals
As per 1.2.1.
Male Wistar rats (bred in Central Animal House of Panjab University, Chandigarh) weighing 180-200 g at the start of the experiment were randomly divided into various groups consisting of 6 - 8 animals per group.
8.2.2.2. Induction and characterization of lipopolysaccharide-induced hypersensitivity

LPS dissolved in normal saline and a single injection of 30 μg/0.1 ml/rat was administered intramuscularly in the left gastrocnemius muscle. Control animals received normal saline 0.1 ml/rat.

8.2.2.3. Behavioral assessment of nociception

Mechanical hyperalgesia (decreased threshold to mechanical stimuli) were evaluated in ipsilateral and contralateral paw of rats. The withdrawal threshold was evaluated using an analgesymeter (Ugo Basile, Italy) by applying noxious pressure to hind paw and expressed in grams as per 1.2.2.3b.

8.2.2.4. Drugs and treatment schedule

Minocycline (Wyeth-Lederle Pharmaceuticals, India) and lipopolysaccharide from Salmonella typhimurium (Sigma, USA) were used in this study. The drug solutions for intraperitoneal administration were freshly prepared by suspending them in one or two drops of Tween 80 in normal saline and administered 1 ml/100 g body weight.

All animals were acclimatized to laboratory environment for at least 2 h before testing. The ipsilateral and contralateral paw withdrawal responses to mechanical stimulation were measured on day 0 (before), 1, 2, 3, 4, 24, 48, 72 h, and 1 week after injecting LPS.

To investigate whether minocycline had any antinociceptive effects, normal animals were administered with two doses of minocycline at 4 h apart between doses and the ipsilateral and contralateral paw withdrawal thresholds were measured for 4 h following last dose of administration.

To investigate whether microglia participate and inhibition of microglial activation able to prevent the development of hyperalgesia following LPS-induced nociception, rats were administered intraperitoneally a single injection of minocycline (10 or 30 mg/kg) 4 h before and another injection of minocycline (10 or 30 mg/kg, i.p.) was administered 30 min before LPS injection and immediately observed for nociceptive behavior after every one hour for four hours and at 24 h. Control animals received only vehicle before LPS injection.

To determine the effect of inhibition of microglial activation on the maintenance of hypersensitivity, a single injection of saline vehicle or minocycline (10 or 30 mg/kg, i.p.) was administered at 20 h after LPS injection and another
injection immediately after behavioral assessment at 24 h after LPS injection to separate group of rats. The paw withdrawal response to mechanical stimulation was tested at 24, 25, 26, 27, 28, and 48 h after LPS injection. Control animals received only vehicle at 20 and 24 h after LPS injection.

8.2.2.5. Statistical analysis
As per 3.1.2.5.

The difference between mean paw withdrawal threshold values of saline- and LPS-injected groups was evaluated by t-test.

8.2.3. RESULTS
8.2.3.1. Induction and characterization of lipopolysaccharide-induced hypersensitivity
In the present series of experiments, the baseline paw withdrawal response in each test obtained on day 0 for each rat was relatively stable and showed no significant variation. The mean paw withdrawal threshold to pressure was 196.67 ± 12.68 g and 191.67 ± 14.33 g, respectively, on day 0 before injecting LPS. The paw withdrawal responses to mechanical stimulation in saline-injected rats remained unchanged from baseline values throughout the entire observation period. Intramuscular administration of LPS (30 µg) produced a marked decrease in ipsilateral paw withdrawal responses from one hour onwards and reached steady state responses between 4 and 48 h after LPS injection indicating the development of hyperalgesia in a timer-dependent manner (Fig. 8.2.1 A). However, the ipsilateral paw withdrawal responses in these animals started restoring to basal levels from 72 h onwards and reached to basal levels 1 wk after LPS administration. Contralaterally, there was similar and significant decrease in paw withdrawal responses to pressure in rats injected with LPS (30 µg), however, the paw withdrawal responses restoring to baseline levels from 72 h onwards after LPS injection (Fig. 8.2.1B).

8.2.3.2. Effect of minocycline on basal paw withdrawal thresholds
Repeated (two dose) administration of minocycline (10 or 30 mg/kg, i.p.) did not produce any change in the ipsilateral and contralateral paw withdrawal thresholds for four hours as compared to respective basal paw withdrawal threshold observed before treatment indicating that minocycline had no antinociceptive effect in normal animals (Fig. 8.2.2A and 2B).

8.2.3.3. Effect of minocycline on development of LPS-induced hyperalgesia

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Repeated (two doses) systemic administration of minocycline (10 or 30 mg/kg, i.p.) 4 h and 30 min before intramuscular administration of LPS in rats significantly attenuated the development of mechanical hypersensitivity in the ipsilateral paw as compared with vehicle-treated rats during the entire observation period (Fig. 8.2.3A). Similarly, the paw withdrawal responses to mechanical stimulation of the contralateral hind paw were also significantly attenuated by repeated administration of minocycline in LPS-injected rats as compared to vehicle-treated LPS-injected rats (Fig. 8.2.3B).

**8.2.3.4. Effect of minocycline on established LPS-induced hyperalgesia**

Repeated (two doses) systemic treatment with minocycline (10 or 30 mg/kg, i.p., 20 and 24 h after LPS injection) had no effect on the hypersensitivity in ipsilateral paw of the LPS-injected rats throughout the observation period as compared with vehicle-treated LPS-injected rats (Fig. 8.2.4A). Further, there was no significant difference in the contralateral paw withdrawal responses to mechanical stimulation after minocycline treatment throughout the observation period as compared to responses of vehicle-treated LPS-injected rats at 24 h (Fig. 8.2.4B).

**8.2.4. DISCUSSION**

In the present study, intramuscular administration of LPS decreased paw withdrawal threshold to mechanical stimulus, which is an indicative of hyperalgesic response. To gain a better sight into the mechanisms involved in the persistent hypersensitivity phenomenon following intramuscular LPS, the present study investigated whether non-neuronal cells, particularly microglia, are activated and involved in induction of hypersensitivity since microglia express receptors for many neurotransmitters and neuromodulators (Palma et al., 1997; Kommers et al., 1998) and can synthesize and release neuroactive factors upon activation, including prostanoids and cytokines (Watkins et al., 2001).

Following repeated systemic administration, minocycline did not affect the paw withdrawal responses in normal animals indicating that minocycline has no intrinsic antinociceptive effects *per se*. However, systemic administration of minocycline, an inhibitor of microglial activation, before LPS injection showed antihyperalgesic effects in rats. Importantly, minocycline markedly attenuated mechanical hypersensitivity in LPS-treated rats. Further, minocycline improved only the decreased paw withdrawal thresholds by LPS to the level of the animals that receive only vehicle treatment. The most striking findings of the present study were
Fig. 8.2.1. Mechanical withdrawal thresholds after a single injection of lipopolysaccharide (LPS; 30μg) into the muscle on the (A) ipsilateral and (B) contralateral paw. Responses at time 0 h represent baseline paw withdrawal responses. Values are mean ± S.E.M. * $P < 0.05$ as compared to saline-injected group. (t-test).
Fig. 8.2.2. Effect of repeated systemic administration of minocycline (10 or 30 mg/kg) on (A) ipsilateral and (B) contralateral paw withdrawal responses to pressure in normal animals. Responses at time 0 h represent baseline paw withdrawal responses. Values are mean ± S.E.M.
Fig. 8.2.3. Effect of repeated administration of minocycline (Min; 10 or 30 mg/kg, i.p.) on the development of mechanical hypersensitivity to pressure in (A) ipsilateral and (B) contralateral paws following intramuscular lipopolysaccharide (LPS) in rats. Responses at time 0 h represent baseline paw withdrawal responses. Values are mean ± S.E.M. *P < 0.05 vs LPS-injected group (one-way ANOVA followed by Dunnett’s test). (n = 6-8 in each group).
Fig. 8.2.4. Effect of repeated administration of minocycline (Min; 10 or 30 mg/kg, i.p.) on the established mechanical hypersensitivity to pressure in (A) ipsilateral and (B) contralateral paws following intramuscular lipopolysaccharide (LPS) in rats. Responses at time 0 h represent baseline paw withdrawal responses. Values are mean ± S.E.M. (n = 6-8 in each group).
those revealing that prior systemic administration of minocycline markedly attenuated LPS-induced contralateral hyperalgesia. The effects of minocycline do not reflect hypoalgesic activity because ipsilateral and contralateral paw withdrawal responses were not affected by systemic administration of minocycline in normal animals. A recent study reported that spinal pre-treatment with minocycline prevented bilateral allodynia in zymosan-induced sciatic inflammatory neuropathy, which is dependent on glial activation and subsequent release of proinflammatory cytokines (Milligan et al., 2003; Ledeboer et al., 2005).

It is well known that minocycline, a second-generation tetracycline, is known to selectively inhibit microglial activation without affecting neurons and astrocytes (Tikka et al., 2001; Tikka and Koistinho, 2001). The lack of effect of minocycline in normal animals to noxious mechanical stimulus despite its antihyperalgesic effect in LPS-injected animals indicates a selective interaction of microglial activation on central nociceptive processing associated with pathophysiological events rather than with normal nociceptive function. A recent study reported that selective inhibition of microglial activation by minocycline attenuated hypersensitivity to thermal and mechanical stimulation following nerve injury and immunological stimulus (Raghavendra et al., 2003a; Ledeboer et al., 2005). Consistent with previous report, the results of the present study well support the role of activated microglia for facilitation of nociceptive processing that occurs during early manifestation of LPS-induced muscle hyperalgesia.

In the present study, post-treatment with minocycline after LPS injection failed to reverse existing hypersensitivity suggesting the differential role of microglia in the development and maintenance of hypersensitivity. It has been hypothesized that microglial and astrocytes have distinct role in hypersensitivity and microglial activation was observed in the induction phase with delayed activation of astrocytes following nerve injury and inflammatory stimuli (Colburn et al., 1997, Coyle, 1998; Svensson et al., 2005b). Recently, it has been reported that there is a sequential activation of ERK in the spinal cord after nerve injury first in neurons, but only for a short period, then in microglia for many days, and finally with a delay in several weeks, in astrocytes (Zhuang et al., 2005). One possibility is that activated microglia contributes to the induction and development of exaggerated pain states and further activates various other cells, particularly astrocytes that are likely to contribute to maintaining hypersensitivity. Of particular relevant to this is the observation that
pinhibition of microglial activation attenuated the development, however, it had no effect on maintenance of hypersensitivity (Raghavendra et al., 2003a; Svensson 2005a). Because, minocycline is without any effect on neurons and astrocytes (Tikka et al., 2001; Tikka and Koistinho, 2001), it seems likely that the attenuation of hypersensitivity could be due to selective inhibition of microglial activation.

Although we did not quantify microglial activation and proinflammatory mediators, previous studies demonstrate that minocycline markedly inhibited p38 MAPK activity and decreased spinal IL-1β and TNF-α expression, both in vitro and in vivo (Yrjanheikki et al., 1998; 1999; Du et al., 2001; Tikka and Koistinaho, 2001; Raghavendra et al., 2003a; Ledeboer et al., 2005). It has been reported that minocycline exerts its effects by inhibiting p38 MAPK, an important regulator of the expression of proinflammatory cytokines and other mediators particularly COX-2 (Kumar et al., 2003). Moreover, minocycline inhibited excitotoxin induced p38 MAPK expression in neuron and mixed spinal cord cultures (Lin et al., 2001; Tikka et al., 2001; Tikka and Koistinaho, 2001). Recent evidences indicate that p38 MAPK expression is increased predominantly in DRG microglia and inhibitors of p38 MAPK reduced hypersensitivity in neuropathic and inflammatory pain (Jin et al., 2003; Schaefer et al., 2003; Svensson et al., 2003; Tsuda et al., 2004). Most importantly, similar to the present findings with minocycline, in these studies p38 MAPK inhibitors prevented but failed to reverse hypersensitivity indicating the pivotal role of microglial activation in the early induction of hyperalgesia. Taken together, these present findings suggest that microglial cells might be continuously activated and release excitatory pain neurotransmitters/neuromodulators that leads to development of hypersensitivity and inhibition of microglial activation early before neuronal excitation and central sensitization to occur could prevent hyperalgesia.

The results of the present study clearly demonstrate that intramuscular administration of LPS induces muscular pain. Further, minocycline attenuation of mechanical hypersensitivity in LPS-injected rats is associated with its ability to inhibit activated microglia. The present results also demonstrate that activated microglia is involved in the development but not maintenance of LPS-induced hypersensitivity and pre-treatment with minocycline started early before microglial activation could prevent the development of hypersensitivity.