Joint manipulation reduces hyperalgesia by activation of monoamine receptors but not opioid or GABA receptors in the spinal cord

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Abstract

Joint manipulation has long been used for pain relief. However, the underlying mechanisms for manipulation-related pain relief remain largely unexplored. The purpose of the current study was to determine which spinal neurotransmitter receptors mediate manipulation-induced antihyperalgesia. Rats were injected with capsaicin (50 μl, 0.2%) into one ankle joint and mechanical withdrawal threshold measured before and after injection. The mechanical withdrawal threshold decreases 2 h after capsaicin injection. Two hours after capsaicin injection, the following drugs were administered intrathecally: bicuculline, blocks γ-aminobutyric acid ($GABA_A$) receptors; naloxone, blocks opioid receptors; yohimbine blocks, α_2-adrenergic receptors; and methysergide, blocks $5\text{-}HT_{1/2}$ receptors. In addition, NAN-190, ketanserin, and MDL-72222 were administered to selectively block $5\text{-}HT_{1A}$, $5\text{-}HT_{2A}$, and $5\text{-}HT_3$ receptors, respectively. Knee joint manipulation was performed 15 min after administration of drug. The knee joint was flexed and extended to end range of extension while the tibia was simultaneously translated in an anterior to posterior direction. The treatment group received three applications of manipulation, each 3 min in duration separated by 1 min of rest. Knee joint manipulation after capsaicin injection into the ankle joint significantly increases the mechanical withdrawal threshold for 45 min after treatment. Spinal blockade of $5\text{-}HT_{1/2}$ receptors with methysergide prevented, while blockade of α_2-adrenergic receptors attenuated, the manipulation-induced antihyperalgesia. NAN-190 also blocked manipulation-induced antihyperalgesia suggesting that effects of methysergide are mediated by $5\text{-}HT_{1A}$ receptor blockade. However, spinal blockade of opioid or $GABA_A$ receptors had no effect on manipulation induced-antihyperalgesia. Thus, the antihyperalgesia produced by joint manipulation appears to involve descending inhibitory mechanisms that utilize serotonin and noradrenaline.

Keywords

Joint manipulation; Serotonin; Noradrenaline; Pain; Capsaicin; Spinal cord

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1. Introduction

Joint manipulation has long been used as a modality for pain relief. The use of this form of manual therapy has evolved from the traditions of bonesetting to orthodox practice in a number of health care disciplines. Modern manipulative therapy can range from slow oscillating glides to high velocity, low amplitude techniques (Haldeman and Hooper, 1999). Manipulation-induced analgesia has been demonstrated in a number of studies in human subjects (Vernon et al., 1990; Vicenzino et al., 1996, 1998; Zusman et al., 1989). Also, recent meta-analyses of the clinical literature, focusing on spinal manipulation, suggest that manipulative therapy is effective for the treatment of acute and chronic musculoskeletal pain (Bronfort, 1999; van Tulder et al., 1997). However, the underlying physiological mechanisms for joint manipulation-related pain relief remain largely unexplored.

It has been suggested that manipulation-induced analgesia may be a multifactorial effect resulting from beneficial influences on the chemical environment of peripheral joints, facilitation of tissue repair processes, segmental inhibitory processes within the central nervous system and activation of descending inhibitory pathways projecting from the brain to spinal cord (Wright, 1995; Wright and Vicenzino, 1995). Recently, we showed that knee joint manipulation decreases secondary mechanical hyperalgesia in the paw induced by injection of capsaicin into the ankle joint in rat (Sluka and Wright, 2001). Since the manipulation is proximal to the injured joint, these data suggest that central neural mechanisms mediate the reduction in hyperalgesia.

Blockade of spinal receptors can elucidate potential mechanisms for the antihyperalgesia produced by joint manipulation. A number of different receptors found in the dorsal horn of the spinal cord may be involved. Presynaptic inhibition can occur through activation of spinal γ-aminobutyric acid (GABA) receptors on primary afferent fibers, which depolarize the terminal thus inhibiting neurotransmitter release from primary afferents and the consequent incoming afferent activity (see Eccles et al., 1962; Sluka et al., 1995). GABA receptors are also located post-synaptically and when activated hyperpolarize the neuron thus decreasing the effectiveness of excitatory input (Malcangio and Bowery, 1996).

Opioids are involved in both segmental inhibition and descending inhibition (Fields and Basbaum, 1999). However, systemic blockade of opioid receptors with naloxone has no effect on the analgesia produced by manipulation in humans (Vicenzino et al., 2000; Zusman et al., 1989). Descending inhibitory pathways from the rostral ventral medulla (RVM) utilize serotonin as a neurotransmitter and those from dorsolateral pons utilize noradrenline (Fields and Basbaum, 1999). The purpose of this study is to determine, using behavioral pharmacology techniques, which dorsal horn neurotransmitter receptors mediate this manipulation-induced antihyperalgesic effect.

2. Methods

All experiments were approved by the Animal Care and Use Committee at the University of Iowa and are in accordance with the National Institutes of Health and the International Association for the Study of Pain policies on use of laboratory animals.

2.1. Joint inflammation

Male Sprague-Dawley rats (250–350 g, Harlan, Indianapolis, IN; $n = 113$) were anesthetized briefly with halothane (2–5% v/v) and injected with 50 μl of 0.2% w/v capsaicin (Sigma, St Louis, MO) into the left ankle joint. This produces a robust secondary mechanical hyperalgesia of the paw, which is fully developed in 2 h after injection (Sluka, 2002).

2.2. Drug administration

Intrathecal catheters (32 G, polyurethane; 10.5 cm; Recathco, Allison Park, PA) were placed acutely immediately prior to drug administration (Storkson et al., 1996). Prior to insertion, a 32-gauge polyurethane catheter was glued to PE 10 tubing (0.28 mm ID, polyethylene; 5 cm; Becton Dickson and Company, Sparks, MD). Animals were anesthetized with halothane (2–5% v/v) and a 23-gauge hypodermic needle was inserted through the skin into the L5—L6 intervertebral space until a tail flick was noted. The 32-gauge polyurethane catheter was inserted into the intrathecal space through the needle and advanced cranially until 3.5–4.0 cm was under the skin. After placement, the needle was withdrawn. Drugs were then given intrathecally, through the catheter, while the animal remained anesthetized, and the catheter removed after drug administration. Acute catheter placement was chosen instead of direct spinal injection in order to insure that the drug was targeted specifically to the lumbar enlargement and the effects of dilution and diffusion on drug concentration at the target site would be minimized. It was not possible, therefore, to verify catheter placement in these experiments with acute catheter placement since the catheter is removed after drug administration. However, the experimenters placing the catheters have performed hundreds of chronic catheter placements with approximate accuracy of 99% (Skyba et al., 2002; Sluka, 2002). Further, the presence of a tail flick upon needle insertion is a good indication that the needle has access to the intrathecal space.

Bicuculline methiodide (0.3 μg/10 μl; $n = 11$), naloxone hydrochloride dihydrate (10 μg/10 μl; $n = 12$), methysergide maleate (30 μg/10 μl; $n = 12$), or yohimbine hydrochloride (30 μg/10 μl; $n = 10$) were administered spinally to block γ-aminobutyric acid ($GABA_A$) receptors (Kaneko and Hammond, 1997), opioid receptors (Dickenson et al., 1981; Woolf, 1980), serotonergic ($5-HT_1$ and $5-HT_2$) receptors (Calejesan et al., 1998), or $α_2$-adrenergic receptors (Sawynok and Reed, 1991), respectively. In addition, 1-(2-methoxyphenyl)-4-(4-[2-phthalimido]butyl)piperazine hydrobromide (NAN-190, 15 μg/10 μl; $n = 12$), ketanserin tartrate (30 μg/10 μl; $n = 14$), or 3-tropanyl-3,5-dichlorobenzoate (MDL-72222, 12 μg/10 μl; $n = 12$) were administered spinally to block $5-HT_{1A}$ (Mjellem et al., 1993), $5-HT_{2A}$ (Sasaki et al., 2001), or $5-HT_3$ receptors (Sasaki et al., 2001), respectively. All doses were chosen based on previously published studies showing selectivity for the specific receptor type/subtype(s), e.g. blocking the analgesic effects of the corresponding receptor agonist. All drugs were purchased from Sigma Chemical Co. (St Louis, MO, USA). Isotonic, sterile saline adjusted to pH 7.2 served as a vehicle control ($n = 12$). Drugs were given 15 min prior to the joint manipulation while the animal was anesthetized with halothane (1–2% v/v). All animals received only one administration of antagonist or vehicle. None of the drugs administered intrathecally had any effect on gross motor function, i.e. gait or withdrawal to noxious stimuli.

In our laboratory, we previously tested yohimbine hydrochloride, methysergide maleate, NAN-190, ketanserin, and MDL-72222 and showed that the doses used in the current study blocked the analgesic effects of appropriate receptor agonists (Radhakrishnan et al., 2003). Also, in the current study, we confirmed that the doses of naloxone hydrochloride dihydrate and bicuculline methiodide blocked the analgesic effects of their agonists. Two hours after intra-articular capsaicin injection, separate groups of animals were pretreated with bicuculline methiodide (0.3 μg/10 μl; $n = 4$), naloxone hydrochloride dihydrate (10 μg/10 μl; $n = 5$), or vehicle (2 groups; $n = 9$). After 15 min, animals in the naloxone group and one of the vehicle groups received i.t. injections of morphine sulfate (1 μg/μl; Sigma, St Louis, MO), while animals in the bicuculline group and the other vehicle group received i.t. injections of muscimol (0.1 μg/10 μl; Sigma, St Louis, MO).

2.3. Joint manipulation

After drug administration, anesthesia was maintained with 1–2% v/v halothane for approximately 30 min. The knee joint manipulation was performed under anesthesia 15 min after drug administration. The femur ipsilateral to the injection site was stabilized, and manipulation was performed by moving the tibia on the femur. The knee joint was flexed and extended to the end range of extension while the tibia was simultaneously translated in an anterior to posterior direction. The treatment group received three applications of manipulation, each 3 min in duration separated by 1 min of rest. Our group has previously shown this time frame to be optimal for producing antihyperalgesia in this model (Sluka and Wright, 2001). Three control groups were utilized: (1) vehicle was given with manipulation; (2) vehicle was given with anesthesia only; and (3) drugs were given with anesthesia only. Previously, we demonstrated that anesthesia with hand contact (sham) has no effect on the hyperalgesia and is similar to anesthesia alone (Sluka and Wright, 2001).

2.4. Behavioral measurements

Animals were tested for withdrawal thresholds to mechanical stimuli (von Frey filaments) applied to the plantar aspect of the hindpaw (Gopalkrishnan and Sluka, 2000). Von Frey filaments with bending forces from 9 to 418 mN were applied in a progressively increasing manner until the hindpaw was withdrawn or 418 mN was reached. Each filament was applied twice. The filament of lowest bending force from which the animal withdrew was considered the mechanical withdrawal threshold of the hindpaw. After a response, the filaments above and below were tested to confirm the withdrawal threshold. The test—retest reliability of this method was previously established ($r^2 = 0.7$; $p = 0.007$) (Gopalkrishnan and Sluka, 2000). Tests were performed before and 2 h after capsaicin injection, and 15, 30, 45 min and 1 h after joint manipulation.

2.5. Statistical analysis

Differences in mechanical withdrawal thresholds between groups were assessed with a Kruskal—Wallis analysis of variance test and a Mann—Whitney U post-hoc test. Values were considered significant at $P \leq 0.05$. All data are presented as the median with the 25th and 75th percentiles.

3. Results

3.1. Joint manipulation reverses mechanical hyperalgesia

Two hours after injection of capsaicin into the ankle joint, there was a decrease in the mechanical withdrawal threshold of the ipsilateral paw for all groups ($P = 0.0001$, signed rank test). Manipulation of the knee joint significantly increased the mechanical withdrawal threshold of the ipsilateral paw 15 min ($P = 0.03$, signed rank test), 30 min ($P = 0.05$, signed rank test), and 45 min ($P = 0.03$, signed rank test) after application (Fig. 1). The decrease in withdrawal threshold returned by 60 min.

Analysis of all groups shows a significant group effect for mechanical withdrawal threshold 15 min ($\chi^2 = 68$, $P = 0.0001$), 30 min ($\chi^2 = 52$, $P = 0.0001$), and 45 min ($\chi^2 = 34$, $P = 0.003$) after joint manipulation. The following results are organized by drug treatment.

3.2. Methysergide prevents manipulation-induced antihyperalgesia

Spinal administration of methysergide maleate (30 μg/10 μl), 15 min before manipulation of the knee joint, prevented the increase in mechanical withdrawal thresholds resulting from the treatment. Withdrawal threshold values for the group pretreated with methysergide were significantly less when compared to the manipulation and vehicle group 15 min ($P = 0.009$),

30 min ($P = 0.041$), and 45 min ($P = 0.026$) after manipulation (Fig. 1A). Methysergide without joint manipulation had no effect on the decreased mechanical withdrawal threshold, and thresholds were similar to intrathecal injection of vehicle without joint manipulation.

3.3. Yohimbine attenuates manipulation-induced antihyperalgesia

Intrathecal administration of yohimbine hydrochloride (30 μg/10 μl) 15 min prior to manipulation of the knee joint attenuated the increase in withdrawal threshold, which results from knee joint manipulation. The withdrawal threshold for the group pretreated with yohimbine was significantly less 15 min ($P = 0.041$) after manipulation when compared to the group that received vehicle prior to manipulation (Fig. 1B). Yohimbine without joint manipulation had no effect on the decreased mechanical withdrawal threshold, and thresholds were similar to intrathecal injection of vehicle under anesthesia without joint manipulation (Fig. 1B).

3.4. Bicuculline or naloxone does not affect manipulation-induced antihyperalgesia

Intrathecal administration of bicuculline methiodide (0.3 μg/10 μl) or naloxone hydrochloride dihydrate (10 μg/10 μl), prior to manipulation of the knee joint, had no effect on the resultant increase in mechanical withdrawal thresholds when compared to vehicle control (Fig. 1C and D). Further, neither drug without joint manipulation affected the decreased mechanical withdrawal thresholds produced by capsaicin, and thresholds were similar to vehicle without joint manipulation (Fig. 1C and D). This suggests that the changes in withdrawal thresholds noted in treatment groups result from joint manipulation and not from administration of the receptor antagonists.

While the doses of bicuculline and naloxone were chosen based on previously published work (Dickenson et al., 1981; Kaneko and Hammond, 1997; Woolf, 1980), we tested them against their agonists in this model to confirm that they were adequate to block spinal $GABA_A$ and opioid receptors, respectively. Administration of either morphine or muscimol in vehicle groups increased mechanical withdrawal thresholds 15 and 30 min after drug injection (Table 1). However, pretreatment with naloxone completely blocked the effect of morphine, and pretreatment with bicuculline completely blocked the effect of muscimol on mechanical withdrawal thresholds (Table 1). Thus, the inability of spinal naloxone and bicuculline to prevent or attenuate manipulation-induced changes in mechanical withdrawal thresholds is not due to ineffective blockade of spinal opioid or $GABA_A$ receptors.

3.5. Effects of selective 5-HT receptor antagonists

Spinal administration of NAN-190 (15 μg/10 μl, $5\text{-}HT_{1A}$) 15 min before knee joint manipulation prevented the increase in mechanical withdrawal threshold resulting from the manipulation. Withdrawal threshold values for the ipsilateral side were significantly less than those for the manipulation and vehicle group 15 min ($P = 0.002$), 30 min ($P = .0\ 015$), and 45 min ($P = 0.002$) after manipulation (Fig. 2A). NAN-190 without joint manipulation had no effect on the decreased mechanical withdrawal threshold, and thresholds were similar to intrathecal injection of vehicle without joint manipulation.

In contrast, spinal administration of ketanserin (30 μg/10 μl, $5\text{-}HT_{2A}$) or MDL-72222 (12 μg/10 μl, $5\text{-}HT_3$) prior to knee joint manipulation had no effect on the increase in mechanical withdrawal threshold resulting from the treatment (Fig. 2B and C). Ketanserin or MDL-72222 also had no effect on the decreased mechanical withdrawal thresholds resulting from intra-articular capsaicin injection, and thresholds were similar to intrathecal injection of vehicle without joint manipulation.

4. Discussion

Blockade of spinal cord serotonin receptors prevents the antihyperalgesia resulting from knee joint manipulation. Also, blockade of α_2-adrenergic receptors in the spinal cord attenuates the antihyperalgesia produced by knee joint manipulation. In contrast, spinal administration of GABA$_A$ or opioid receptor antagonists does not affect manipulation-induced antihyperalgesia in this animal model. Further, selective serotonin receptor antagonists were administered to determine the serotonin receptor subtype (s) involved in this antihyperalgesia. Blockade of spinal 5-HT$_{1A}$ receptors, but not 5-HT$_{2A}$ or 5-HT$_3$ receptors, prevents manipulation-induced antihyperalgesia. These data suggest that knee joint manipulation activates descending inhibitory pathways that utilize serotonin and noradrenaline, which inhibit transmission of nociceptive information by acting on 5-HT$_{1A}$ and α_2-adrenergic receptors in spinal cord of rats.

It should be noted that this study was designed so that an uninjured joint, proximal to the site of injury was manipulated. This manipulation is within the normal working range of the joint that activates large diameter joint afferents in the uninjured knee joint (see Schaible and Grubb, 1993). Thus, manipulation of the uninjured knee is expected to activate large diameter afferent fibers to reduce hyperalgesia.

4.1. Descending monoaminergic inhibitory pathways

Serotonergic fibers in the spinal cord originate supraspinally in the RVM whereas noradrenergic fibers in the spinal cord originate supraspinally in the dorsolateral pons (Clark and Proudfit, 1991; Fields and Basbaum, 1999; Westlund et al., 1983). Agonists to serotonergic and α_2-adrenergic receptors delivered spinally produce analgesia, reduce hyperalgesia, and reduce dorsal horn neuron activity (Ali et al., 1994; Barasi and Clatworthy, 1987; Bardin et al., 2000, 2001; Eide, 1992; Fairbanks and Wilcox, 1999; Oyama et al., 1996; Sawynok and Reed, 1991; Yang et al., 1994; Zhao and Duggan, 1988).

The periaquaductal gray (PAG) sends projections to both RVM and dorsolateral pons (A6/A7 cell groups) (Beitz, 1982; Cameron et al., 1995). From there neurons in both RVM and dorsolateral pons project to the spinal cord (Clark and Proudfit, 1991; Fields and Basbaum, 1999; Skagerberg and Bjorklund, 1985; Sluka and Westlund, 1992; Westlund et al., 1983). There is also evidence that activation of neurons in the RVM, some of which contain substance P, produces spinal antinociception indirectly through connections with descending noradrenergic fibers in the A7 cell group (Nuseir et al, 1999; Yeomans and Proudfit, 1990, 1992). Noradrenaline is the primary neurotransmitter of neurons in the dorsolateral pons and serotonin of neurons in the RVM (Clark and Proudfit, 1991; Fields and Basbaum, 1999; Skagerberg and Bjorklund, 1985). In fact, stimulation of PAG or RVM increases release of 5-HT and noradrenaline spinally (Bowker and Abhold, 1990; Cui et al., 1999; Hammond et al., 1985; Sorkin et al., 1993). Electrical stimulation of any of these descending inhibitory pathways exerts inhibitory effects on dorsal horn neurons (Fields et al., 1977) and produces antinociception (Aimone et al., 1987; Basbaum et al., 1976; Fields and Basbaum, 1999; Jones and Gebhart, 1986; Mayer et al., 1971; Reynolds, 1969; Yeomans et al., 1992). In the current study, spinal administration of methysergide or yohimbine prevents or decreases joint manipulation-induced antihyperalgesia. Since serotonin and noradrenaline in spinal cord are released from descending bulbospinal neurons, our data strongly support the hypothesis that joint manipulation-induced antihyperalgesia involves activation of descending inhibitory pathways. However, since naloxone has no effect on joint manipulation-induced antihyperalgesia, this suggests activation of non-opioid descending inhibitory systems. Further, blockade of GABA receptors in the spinal cord has no effect on joint manipulation-induced antihyperalgesia, suggesting that presynaptic or local inhibitory pathways are likely not involved in joint manipulation antihyperalgesia.

The role of this descending analgesia system in modulating pain-related behavior may depend on duration and type of noxious stimuli involved. Descending serotonergic neurons do not appear to tonically inhibit spinal transmission of noxious mechanical or thermal input in normal animals (Bardin et al., 2000; Zhuo and Gebhart, 1991). In contrast, spinal administration of noradrenergic receptor antagonists decreases hotplate and tail flick latencies in rat, indicating that descending noradrenergic neurons are involved in tonic inhibition of spinal transmission of noxious heat (Proudfit and Hammond, 1981; Sagen and Proudfit, 1984; Zhuo and Gebhart, 1991). There is also pharmacological evidence suggesting both descending serotonergic and noradrenergic pathways are activated in models of chemogenic and neuropathic pain. Intrathecal pretreatment with methysergide or yohimbine increases licking and biting during both phases of the formalin test and post-treatment increases pain-related behaviors in the second phase (Omote et al., 1998). Spinal administration of methysergide or yohimbine also further reduces paw withdrawal latencies to heat after chronic constriction injury (Satoh and Omote, 1996). Further, dorsal horn serotonin and noradrenaline concentrations increase bilaterally after formalin injection and after chronic constriction injury (Omote et al., 1998; Satoh and Omote, 1996). In the current study, spinal administration of serotonin or noradrenaline receptor antagonists does not further reduce mechanical withdrawal thresholds subsequent to intra-articular capsaicin injection. Thus, descending monoaminergic neurons do not appear to inhibit spinal transmission of mechanical stimuli (mechanical hyperalgesia) after capsaicin injection.

4.2. Selective serotonin receptor antagonists

In this study, spinal administration of methysergide prevents joint manipulation-induced antihyperalgesia. The effectiveness of this non-selective serotonin receptor antagonist prompted evaluation of more selective serotonin receptor antagonists. The 5-HT$_1$, 5-HT$_2$, and 5-HT$_3$ types of serotonin receptors are found in the spinal dorsal horn (Kia et al., 1995; Ridet et al., 1994; Thor et al., 1993, see Coggeshall and Carlton, 1997 for review). However, there is some controversy regarding the role of serotonin receptors in spinal antinociception. For instance, spinal administration of 5-HT$_{1A}$ receptor agonists facilitates nociceptive transmission within the spinal cord (Alhaider and Wilcox, 1993; Ali et al., 1994; Zhang et al., 2001b), inhibits spinal nociceptive transmission (Bardin et al., 2001; Mjellem et al., 1992; Oyama et al., 1996), or has no effect (Bardin et al., 2000; Millan, 1994; Sasaki et al., 2001). In the present study, spinal administration of methysergide or NAN-190 prevents antihyperalgesia induced by knee joint manipulation. This suggests that peripheral joint manipulation increases physiological levels of serotonin that predominantly act on spinal 5-HT$_{1A}$ receptors to produce antihyperalgesia.

The central neural mechanisms by which peripheral nonpharmacological manipulations like transcutaneous electrical nerve stimulation (TENS) or manipulative therapy relieve pain are pharmacologically heterogeneous. For instance both low and high frequency TENS decrease secondary hyperalgesia produced by injection of 3% kaolin/carrageenan into the knee joint (Sluka et al., 1998,1999). However, the mechanisms for TENS-antihyperalgesia differ depending upon frequency of stimulation. Antihyperalgesia produced by low (4 Hz) and high (100 Hz) frequency TENS is dependent upon spinal μ- and δ-opioid receptors, respectively (Sluka et al., 1999), while data from the current study show that antihyperalgesia induced by peripheral knee joint manipulation, administered at a frequency of 0.5–1 cycles per second, does not depend on spinal opioid receptors. Further, blockade of spinal 5-HT$_2$ or 5-HT$_3$ receptors prevents antihyperalgesia induced by low, but not high, frequency TENS, while blockade of spinal α$_2$-adrenergic receptors with yohimbine has no effect (Radhakrishnan et al., 2003). In contrast, our data show that spinal blockade of 5-HT$_{1A}$ and α$_2$-adrenergic receptors prevents or attenuates antihyperalgesia resultant from knee joint manipulation. These findings should be tempered by the fact that the pain model employed may affect serotonin receptor

expression. For instance, carrageenan inflammation is associated with upregulation of 5-HT_{2A} mRNA and increases in extracellular 5-HT in the dorsal horn of the spinal cord (Zhang et al., 2000,2001a).

4.3. Manipulation-induced antihyperalgesia

Manipulation of cervical or thoracic spine produces an immediate, localized hypoalgesic effect and reduces pain in human subjects (Terrett and Vernon, 1984; Vernon et al., 1990; Vicenzino et al., 1996; Zusman et al., 1989). Further, cervical manipulation produces hypoalgesia in, people with lateral epicondylitis or cervical spine pain, that is accompanied by sympathoexcitation: evidenced by changes in skin conductance, blood flow and/or skin temperature (Sterling et al., 2001; Vicenzino et al., 1998). In addition, the initial hypoalgesic effect of spinal manipulation is not reversed by systemic administration of naloxone (Vicenzino et al., 2000; Zusman et al., 1989). Wright and colleagues hypothesize that this coordinated hypoalgesic and autonomic response may be the result of activation of descending inhibitory pathways in the central nervous system. Specifically they suggest that joint manipulation activates the lateral periaqueductal gray since glutamate excitation of the lateral PAG produces a similar response, namely that of non-opioid analgesia, sympathetic excitation, and motor facilitation (Bandler and Shipley, 1994; Cannon et al., 1982; Lovick, 1991). These data from human subjects are consistent with the current findings in this animal model. Taken together, we hypothesize that joint manipulation produces a non-opioid form of analgesia, mediated by spinal serotonergic and noradrenergic receptors utilizing descending inhibitory pathways from the RVM and dorsolateral pons.

Although manipulation in clinical settings is often performed at the site of injury or the spine, a segmentally related but non-involved joint was chosen in order to minimize local peripheral effects and focus on those effects mediated by the central nervous system. Further studies are necessary to more directly investigate supraspinal involvement and evaluate other pain models. As the time/force profiles of different forms of manipulation vary, other forms of joint manipulation should also be evaluated.

Acknowledgements

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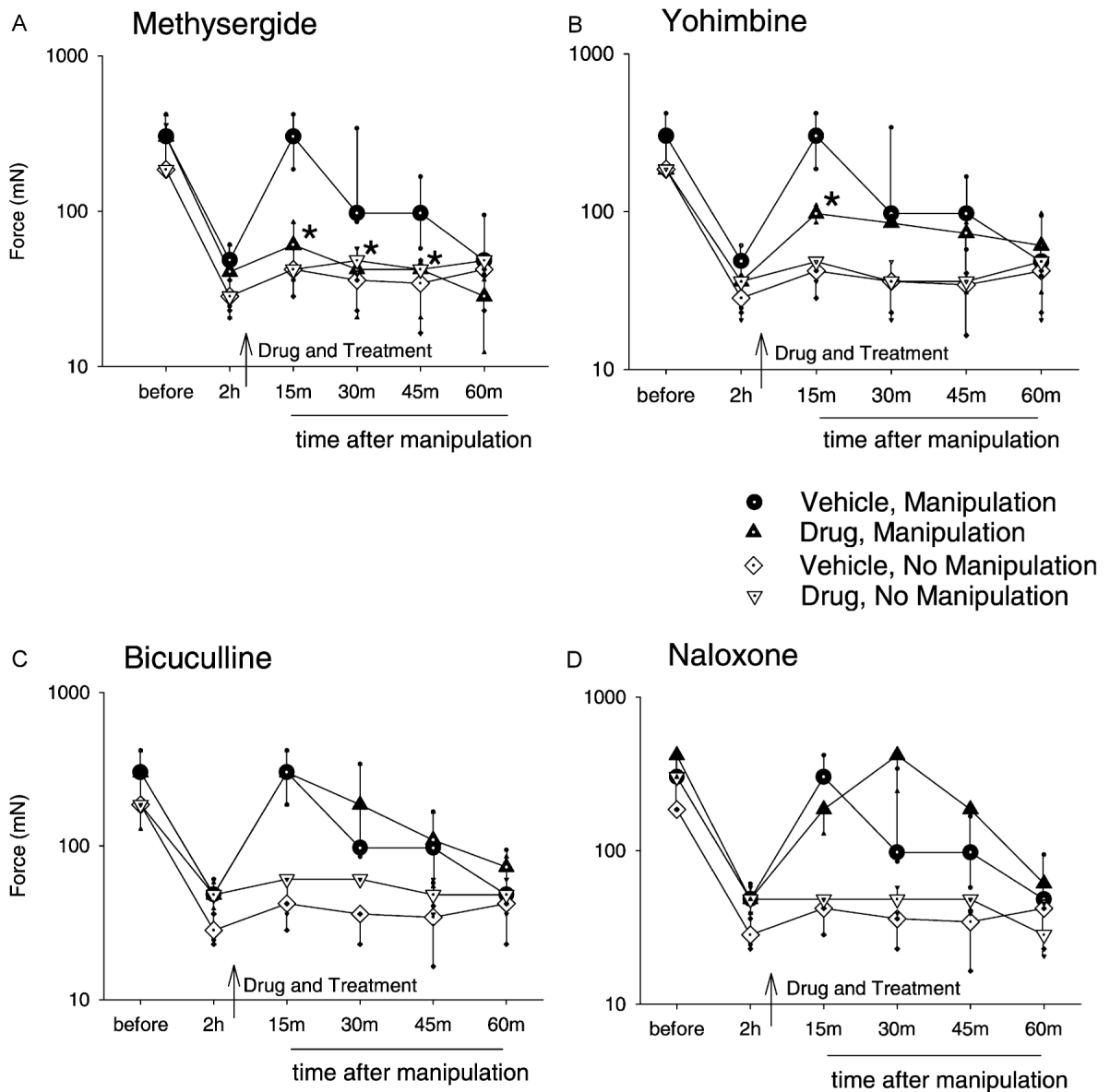
Fig. 1.
Graphs represent the mechanical withdrawal threshold for groups of animals that received drug or vehicle and subsequently knee joint manipulation or no treatment. Separate groups received intrathecal (i.t.) injection of methysergide (A), yohimbine (B), bicuculline (C), or naloxone (D) 2 h after 0.2% intra-articular capsaicin and 15 min prior to joint manipulation. Spinal injection of methysergide (30 μg/10 μl) significantly prevented the manipulation-induced increase in mechanical withdrawal threshold at 15, 30, and 45 min compared with the 'vehicle, manipulation' control group. Yohimbine (30 μg/10 μl) had a partial effect, decreasing the mechanical withdrawal threshold 15 min post-manipulation. Neither i.t. bicuculline (0.3 μg/10 μl) nor naloxone (10 μg/10 μl) significantly affected the increase in mechanical withdrawal threshold resulting from knee joint manipulation. Data are presented as medians with 25th and 75th percentiles. *Significantly less than manipulation with vehicle control ($P < 0.5$).
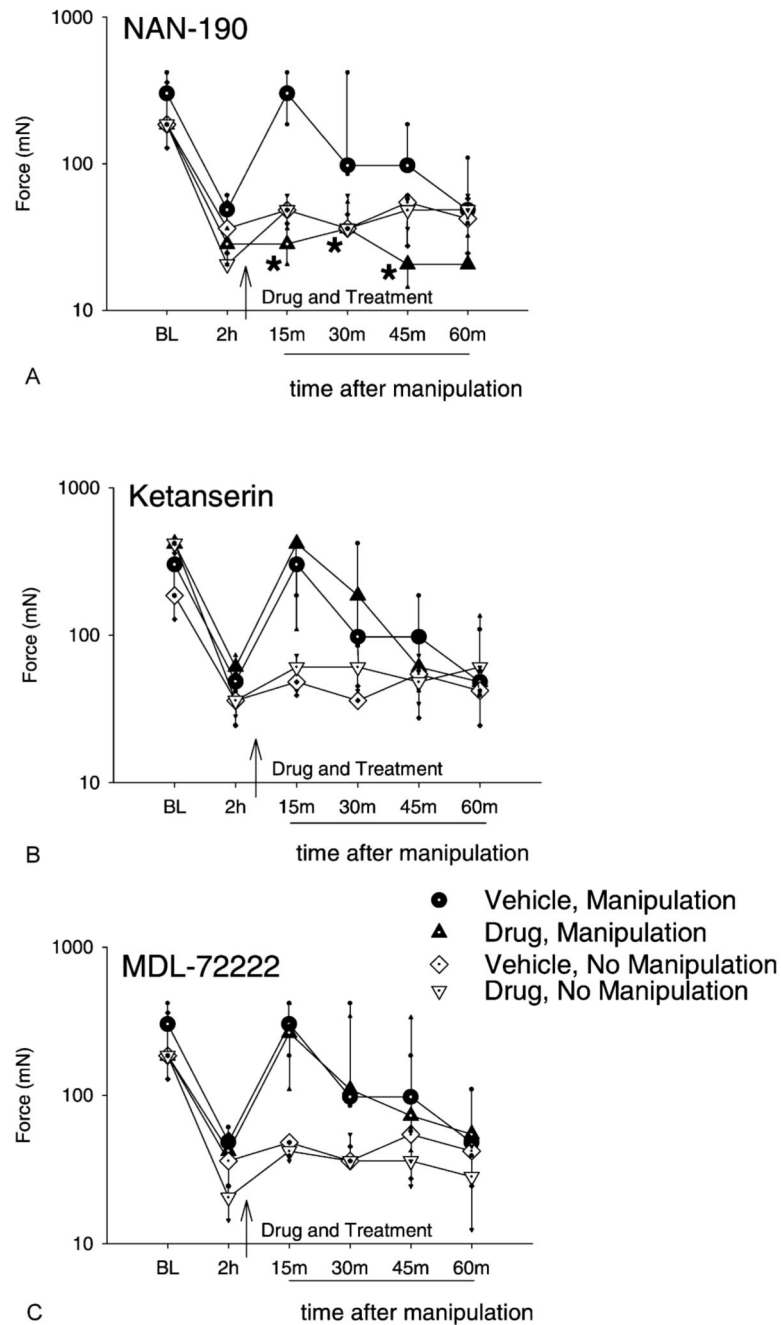
Fig. 2.
Graphs represent the mechanical withdrawal thresholds for groups of animals that received intrathecal (i.t.) injection of the 5-HT$_{1A}$ antagonist, NAN-190 (A), the 5-HT$_{2A}$ antagonist, ketanserin (B), or the 5-HT$_3$ antagonist, MDL-72222 (C) 2 h after 0.2% intra-articular capsaicin and 15 min prior to joint manipulation. Spinal administration of NAN-190 (30 μg/10 μl, 5-HT$_{1A}$) significantly prevented the manipulation-induced increase in mechanical withdrawal threshold at 15 min, 30 min, 45 min compared with the 'vehicle, manipulation' control group. Neither i.t. ketanserin (30 μg/10 μl, 5-HT$_{2A}$) nor MDL-72222 (12 μg/10 μl, 5-HT$_3$) significantly affected the increase in mechanical withdrawal threshold resulting from knee joint

manipulation. Data are presented as medians with 25th and 75th percentiles. *Significantly less than manipulation with vehicle control ($P < 0.05$).

Table 1

Data are presented as the median with 25th and 75th percentiles (mN)

	Morphine (1 µg/10 µl)		Muscimol (0.1 µg/10 µl)	
	15 min	30 min	15 min	30 min
Vehicle (10 µl)	302 (185, 418)	185 (110, 418)	302 (185, 418)	185 (160, 244)
Naloxone (10 µg/10 µl)	36 (21, 39)*	48 (48, 48)*	NA	NA
Bicuculline (0.3 µg/10 µl)	NA	NA	54 (45, 61)*	61 (55, 61)*

*
Significantly different from vehicle ($P < 0.05$).