Original Article

Dexmedetomidine Alleviates Presurgical Anxiety-Induced Persistent Postsurgical Pain via Decreasing the Expression of Glucocorticoid Receptor

Lu Li, Zuo-Xia Zhang, Zhi-Yu Yin, Cui-E Lu, Yi-Shan Lei, Rao Sun, Yue Liu, Zheng-Liang Ma[*], and Xiao-Ping Gu[*]

From Department of Anesthesiology, Affiliated Drum Tower Hospital of Medical School of Nanjing University, Nanjing, China.

Correspondence to Dr. Zheng-Liang Ma at mazhengliang1964@nju.edu.cn or Dr. Xiao-Ping Gu at xiaopinggu@nju.edu.cn.

ABSTRACT

Background: Preoperative anxiety can worsen postsurgical pain. Glucocorticoids play an important role in psychological anxiety and pain, but the effect of glucocorticoid receptor (GR) on persurgical anxiety-induced persistent postsurgical pain remains unknown.

Methods: Adult male Sprague Dawley rats were randomly divided into 10 groups: control group, SPS group, incision group, 'SPS-plus-incision' group, saline group, metyrapone group, Dexmedetomidine group 1 (10 μg/kg), Dexmedetomidine group 2 (20 μg/kg), Dexmedetomidine group 3 (40 μg/kg) and Dexmedetomidine + Corticosterone group. Single-prolonged stress (SPS) was used to induce anxiety behaviors. Intraperitoneal injection of saline and dexmedetomidine was performed at 24 h after SPS and 0.5 h before incision. Intraperitoneal injection of metyrapone (25 mg/kg) was performed at 1h before SPS. Paw withdrawal mechanical threshold (PWMT) was tested at 24 h before SPS and on 1, 4, 7, 14, 21, and 28 days after incision. Corticosterone levels were determined using ELISA. The expression of GR was determined using Western blot.

Results: The 'SPS-plus-incision' group decreased PWMT compared with control group and incision group from 1 to 28 days ($P<0.05$). SPS combined with incision increased plasma corticosterone levels compared with control group ($P<0.05$). A time-dependent increase in GR was also observed in 'SPS-plus-incision' group ($P<0.05$). Metyrapone significantly blunted the SPS-induced persistent postsurgical pain ($P<0.05$). Intraperitoneal administration of dexmedetomidine inhibits SPS-induced persistent pain compared with group saline ($P<0.05$). The expression of GR decreased after the intraperitoneal administration of dexmedetomidine ($P<0.05$). Pretreatment with corticosterone blocked this effect.

Conclusions: Glucocorticoids contributed to presurgical anxiety-induced persistent postsurgical pain. Dexmedetomidine that decreased the expression of GR alleviated anxiety-induced persistent pain. These results indicated that dexmedetomidine may be an effective agent for preventing presurgical anxiety-induced persistent postoperative pain. (Funded by the National Natural Science Foundation of China and the Department of Health of Jiangsu Province, China.)

Persistent postsurgical pain (PPSP) develops after a surgical procedure and is a major clinical problem (1, 2). Previous studies have shown that socioenvironmental and psychosocial risk factors are correlated with the development of persistent postsurgical pain (3, 4). Presurgical anxiety has been identified as a major psychosocial predictor of postsurgical pain and has been shown to exacerbate and prolong postsurgical pain (5, 6). Although this is a major clinical problem, little is known regarding the cellular and molecular mechanisms underlying the effect of anxiety on persistent postsurgical pain, and consequently, relatively few effective therapeutic approaches exist.

Animal studies concerning the effect of psychosocial factors on pain had not been conducted until recently. In an animal model, single-prolonged stress (SPS) was shown to induce anxiety-like behaviors (7). Our previous study showed that SPS combined with plantar incision can mimic clinical presurgical anxiety- induced chronic postoperative pain (8). The results showed that presurgical anxiety induces chronic postoperative pain, but the mechanism behind that induction remains unclear.

Stressful events, such as anxiety, stimulate the hypothalamus-pituitary-adrenal axis (HPA) and, consequently, elevate the release of glucocorticoids (mainly cortisol in humans and corticosterone in rodents) (9). During anxiety, glucocorticoid receptor (GR) is progressively activated by glucocorticoids (10). Furthermore, glucocorticoids exacerbate pain-like behaviors (11). It has been reported that glucocorticoid receptors are increased in spinal cord dorsal horn neurons after peripheral nerve injury (12). Because glucocorticoids are implicated in both psychological anxiety and pain through activation of glucocorticoid receptors, we hypothesized that glucocorticoids contribute to the development of anxiety-induced hyperalgesia.

A clinical study showed that intraoperative administration of dexmedetomidine reduces the stress response by reducing the plasma levels of cortisol (13). Dexmedetomidine, a highly selective agonist of α2-adrenergic receptors, has sedative, anxiolytic and analgesic effects (14, 15). Additionally, intranasal administration of dexmedetomidine before anesthesia has been reported to alleviate presurgical anxiety (16). Corticosterone also influences anxiety-like behaviors and decreases α2 expression, which may lead to the activation of the HPA. Furthermore, an experiment in an animal model of PTSD showed that dexmedetomidine reduces anxiety-like behaviors and improves cognitive impairments, but the mechanisms behind these effects are unclear (17). However, the effect of dexmedetomidine on presurgical anxiety-induced persistent postoperative pain has not yet been studied. Therefore, in the present study, we investigated the role of glucocorticoids in a rat model of SPS-plus-incision. We hypothesized that dexmedetomidine would alleviate the persistent postoperative pain induced by presurgical anxiety via decease expression of GR. Our results indicated that dexmedetomidine may be an effective pharmacological agent for the prevention of presurgical anxiety-induced persistent postoperative pain.

METHODS

Animals and Ethics Statement

Adult male Sprague Dawley rats weighing 180-220 g were provided by the Laboratory Animal Center of Drum Tower Hospital for use in this study. All rats were housed in an environment with a 12-h light/dark schedule and a temperature of $22 \pm 2 ℃$. The rats were housed in groups of six per cage with food and water available ad libitum. All efforts were made to minimize animal suffering and reduce the number of animals used in this study. All experimental protocols were approved by the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Each behavioral test group contained 8 rats, and each molecular detection group contained 3 rats.

Drug Administration

The following drugs were used in this study: metyrapone (batch number: 856525MSDS, Ju Xionghua Co, China), sevoflurane (batch number: 11123131, Heng Rui Co, China), dexmedetomidine (batch number: 10061468, Xin Chen Co, China), and corticosterone (batch number: D0505AS, Shen ge wei Co, China). Metyrapone was dissolved in 0.9% sterile saline to obtain the final volume of 0.2 ml. Intraperitone-

al injection of metyrapone (25 mg/kg) was performed at 1 hour before the SPS procedure. Dexmedetomidine was dissolved in saline to a volume of 0.2 ml. Corticosterone was dissolved in 10% ethanol solution and was injected intraperitoneally in a 0.2 ml volume. Dexmedetomidine and corticosterone were administered at 24 hours after the SPS procedure and 30 minutes before surgical incision.

SPS

In the SPS model, rats were first immobilized for 2 hours in acrylic animal holders (18). Then, the rats were immediately subjected to a forced swim in a plexiglass cylinder two-thirds filled with fresh water (24℃). The rats were dried, and after a 15-min rest, the rats were exposed to the inhalation anesthetic sevoflurane until loss of consciousness.

Hindpaw Incision Surgery

On the first day after the SPS procedure, an incision was made as previously described (19). Each rat was anesthetized with sevoflurane via a nose mask. A 1-cm longitudinal skin incision was made on the plantar surface of the right hindpaw, starting at 0.5 cm from the edge of the heel. The underlying plantaris muscle was elevated and incised longitudinally, but its origin and insertion were left intact. After hemostasis was achieved with slight pressure, the incision was sutured with interrupted horizontal mattress sutures of 5-0 nylon. Following the incision, the rats were allowed to recover in the home cage for 1 hour.

Mechanical Pain Behavioral Testing

Mechanical pain was assessed using a Dynamic Plantar Aesthesiometer (an automatic von Frey system). Each rat was placed in a transparent Plexiglas compartments (20 cm × 25 cm × 15 cm) on a metal mesh floor and were allowed to acclimatize to the environment for 30 minutes. A metal wire was pressed vertically against the plantar surface and the force was increased uniformly (the force was uniformly increased to 50 g over 15 seconds). A positive response was defined as paw flinching or withdrawal of the hindpaw. The test was repeated five times with a 5-min interval between each application of force.

All rats underwent a behavioral test on day 1 before the SPS procedure and on days 1, 4, 7, 14, 21, and 28 after surgical incision (n=8).

Determination of Serum Corticosterone

Peripheral blood was extracted from the rats via the tail vein 1 day before the SPS procedure and on days 1, 4, 7, 14, and 28 after surgical incision. For each rat, 30 μl of blood was collected, and the procedure took approximately 1 minute. The plasma samples were stored at -20℃ until use. Serum corticosterone levels were determined using a commercially available enzyme competitive ELISA test kit following the manufacturer's instructions (Cayman, USA).

Western Blotting

The rats were anesthetized with sevoflurane (5%) and then quickly sacrificed. The L4-L5 segment of the spinal cord was removed quickly and stored in liquid nitrogen. Tissue samples were homogenized in lysis buffer. The homogenate was centrifuged at 13,000 rpm for 5 minutes at 4℃, and the supernatant was transferred to a new tube. The protein samples were quantified by the Bradford method and were stored at -80℃. SDS-polyacrylamide gel electrophoresis (6%) was used to separate 70 μg of the protein samples, and the separated proteins were transferred to a nitrocellulose membrane at 200 mA for 2 hours using a wet blotting system. The membranes were blocked with PBS/5% skim milk/0.1% Tween 20 for 1 hour at room temperature. Then, the filter membranes were incubated with primary antibody against GR (1:500, Abcam, USA) overnight. Subsequently, the membranes were washed with TBST buffer and incubated with suitable secondary antibody conjugated with horseradish peroxidase (1:5000; Jackson Immuno Research, USA) for 2-3 hours at room temperature. Immune materials were detected using the ECL system (Santa Cruz Biotechnology, CA, USA) followed by film exposure for 1-10 minutes. β-actin was used as the loading control for total protein. The protein bands were quantified by densitometric analysis using an imaging analysis system (Quantity One analysis software (Bio-Rad, Hercules, CA, USA).

Statistical Analyses

SPSS 17.0 software was used to conduct all sta-

tistical analyses. All data are expressed as the means ± standard deviation. All rats were assigned to the treatment groups in a randomized manner. Repeated measures analysis of variance (ANOVA) followed by the Bonferroni post hoc test was used to determine differences in PWMT at each time point. Differences in corticosterone levels were analyzed using ANOVA followed by pairwise comparisons using the Bonferroni t test. One-way ANOVA was used to determine differences in the expression of GR between the experimental groups. Post hoc analysis was performed for multiple comparisons. The statistical significance was set at $P<0.05$.

RESULTS

Experiment 1: SPS Induces an Increase in Plasma Corticosterone Levels and Persistent Mechanical Pain in Plantar Incision Rats

In rats of the control group and 'SPS-plus-incision' group, plasma corticosterone levels were measured by ELISA. Compared with the control group, the plasma corticosterone levels of the 'SPS-plus-incision' group were increased on day 1 after plantar incision, and they remained elevated throughout the 28-day follow-up period after plantar incision ($P<0.05$) (Figure 1).

To determine the effect of SPS on incisional pain, PWMT in the right hindpaw was measured before the SPS procedure and on days 1, 3, 7, 14, 21 and 28 after the surgical incision (n=8). The basal pain thresholds of all experimental groups were equivalent. Compared to the control group (no SPS or incision), plantar incision decreased PWMT from days 1 to 7 after surgery ($P<0.05$). SPS exposure alone decreased PWMT in the right hindpaw compared to the control group from days 1 to 14 after the SPS procedure ($P<0.05$). The 'SPS-plus-incision' group underwent incision surgery at 24 hours after the SPS procedure and exhibited a significantly decreased PWMT compared to the incision alone group ($P<0.05$) from 1 to 28 days after surgery (Figure 2A).

Experiment 2: Glucocorticoids Contribute to SPS-Induced Persistent Mechanical Pain after Plantar Incision
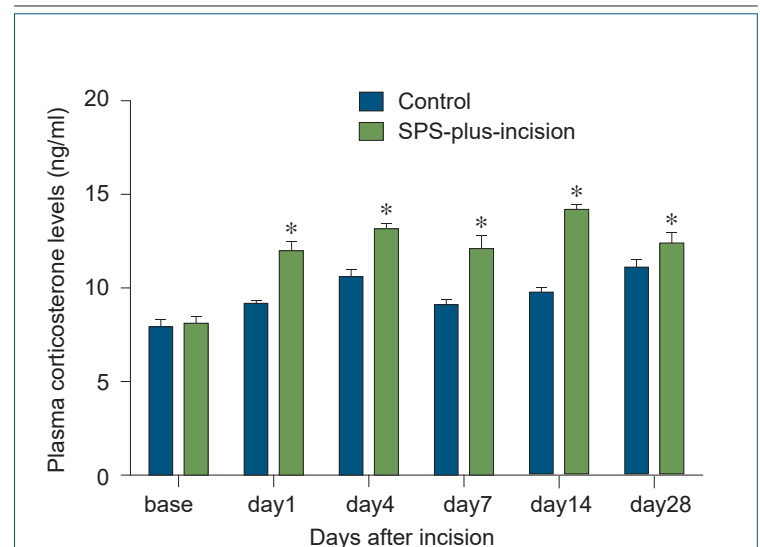
To further investigate the involvement of gluco-

Figure 1. SPS Increased Plasma Corticosterone Levels in Plantar Incision Rats (n=3 for Each Group).
SPS combined with incision increased plasma corticosterone levels from day 1 to day 28 after plantar incision compared with the control group. *$P<0.05$ compared with group C.

corticoids in SPS-induced persistent incisional pain, rats were intraperitoneally injected with 25 mg/kg metyrapone, a corticosterone synthesis inhibitor, before surgical incision. SPS-exposed rats treated with metyrapone exhibited a significant increase in PWMT in the right hindpaw form days 1 to 28 after surgery compared to those treated with saline ($P<0.05$) (Figure 2B). Metyrapone significantly blunted the SPS-induced exacerbation and prolongation of incisional pain in the right hind paw. This suggests that glucocorticoids contributed to the process of mechanical pain induced by SPS.

Experiment 3: Intraperitoneal Administration of Dexmedetomidine Attenuates Anxiety-Induced Persistent Pain

To examine the effect of dexmedetomidine on anxiety-induced persistent pain, 3 different doses of dexmedetomidine were administrated at 24 hours after the SPS procedure and 30 minutes before surgical incision. An injection of 10 μg/kg dexmedetomidine did not affect PWMT (Figure 3A), compared with 'SPS-plus-incision' group. Both 20 μg/kg ($P<0.05$) and 40 μg/kg ($P<0.01$) injections of dexmedetomidine increased PWMT, and this effect was greater for
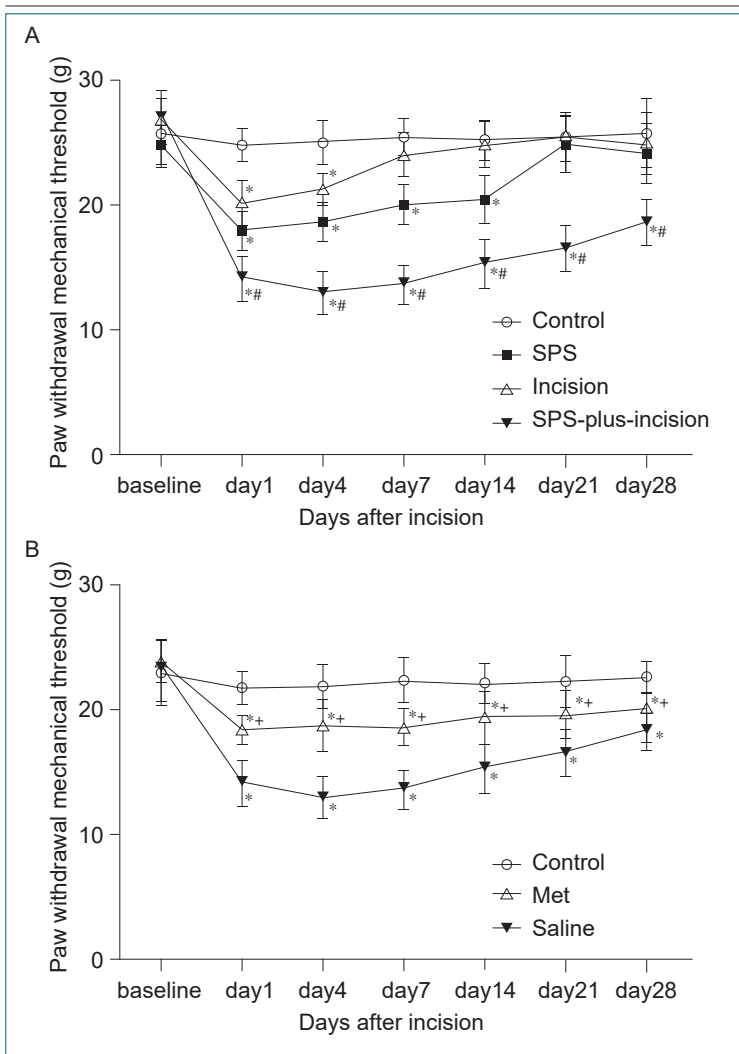
Figure 2. Effects of SPS Exposure and Administration of Metyrapone on PWMT in the Right Hindpaw (n=8 for Each Group).
A. Incision-induced pain lasted for 4 days after plantar incision. Single-prolonged stress alone produced a decrease in PWMT from days 1 to 14 compared to the control group. SPS exacerbated and prolonged incisional pain and significantly decreased PWMT in the 'SPS-plus-incision' group for 28 days after plantar incision; B. For the rats exposed to SPS and plantar incision, those treated with metyrapone exhibited significantly increased PWMT, indicating that metyrapone inhibited SPS-induced persistent mechanical pain after plantar incision. $^*P<0.05$ compared with group C, $^\#P<0.05$ compared with incision group and $^+P<0.05$ compared with saline group.

ies of the potential mechanisms of this effect of dexmedetomidine were carried out using solely the 40 µg/kg dose.

Experiment 4: Dexmedetomidine Ameliorates Anxiety-Induced Persistent Pain by Decreasing the Expression of GR

To further explore the potential mechanisms, we examined whether glucocorticoids are involved in the analgesic effect of dexmedetomidine. Rats were or were not pretreated with 40 µg/kg dexmedetomidine and 10 µg/kg corticosterone at 1 day after the SPS procedure and 30 minutes before plantar incision surgery, and PWMT was not significantly different between the pretreated (group Dex 40 µg/kg + Cort 40 µg/kg) and non-pretreated group (group saline) (Figure 3B). This result indicates that dexmedetomidine-induced pain inhibition is blocked by corticosterone ($P<0.05$). Therefore, dexmedetomidine alleviates SPS-induced persistent pain, possibly via the inhibition of glucocorticoids. To determine the role of glucocorticoids in SPS-induced persistent pain and the analgesic effect of dexmedetomidine, the expression of GR in the dorsal horn was assessed by western blot before and at 1, 7, and 28 days after plantar incision. A time-dependent increase in GR expression was observed in the 'SPS-plus-incision' group, but not in the control group, from 1 to 28 days after paw incision ($P<0.05$) (Figure 4A). Compared to the rats that received saline, the expression of GR decreased significantly after the intraperitoneal administration of dexmedetomidine ($P<0.05$) (Figure 4B-D). However, pretreatment with corticosterone inhibited the down-regulation of GR by dexmedetomidine (Figure 4B-D).

DISCUSSION

Persistent postsurgical pain is intractable, and the multitude of patients suffering from it generate huge economic costs (20). Presurgical anxiety is closely related to postsurgical pain complaints. However, studies concerning this clinical issue are limited, highlighting the need for studies on potential therapeutic strategies for persistent postsurgical pain. The present study showed that SPS-exposure increased incisional pain from days 1 to 28 after incision surgery,

the 40 µg/kg dose at all time points (Figure 3A). These data suggest that the intraperitoneal administration of dexmedetomidine inhibits SPS-induced persistent pain, with higher doses of dexmedetomidine having greater effects. Accordingly, the subsequent behavioral and cellar studies

and this was accompanied by increases in circulating corticosterone concentration and spinal cord GR expression. Treatment with metyrapone before SPS exposure significantly mitigated the anxiety-induced persistent pain, confirming that increases in glucocorticoids contribute to the effects of presurgical anxiety on persistent postoperative pain. Furthermore, we demonstrated that dexmedetomidine ameliorated anxiety- induced persistent pain in rats, probably through decreasing the expression of GR. Posttraumatic stress disorder (PTSD) is an anxiety disorder that develops after exposure to life-threatening trauma, and it has comorbidities that are also observed with many chronic pain conditions (21, 22). SPS is a well-recognized animal model of PTSD that mimics the physiological and behavioral changes that occur in patients who endure high levels of presurgical anxiety. In addition, paw incision is an established animal model that mimics acute postsurgical pain. In this animal model, the incision-induced pain only lasted 7 days. The present results support and expand upon those of our earlier study, which confirmed that incision-induced pain was exacerbated and lasted for 28 days in rats subjected to SPS. The SPS-plus-plantar incision rat model allows researchers to broadly study the impact of anxiety on pain responses and the underlying mechanisms involved.

Glucocorticoids play a key role in neuroplasticity within the central nervous system (23). Previous studies have shown that the presence of glucocorticoids in the central amygdaloid nucleus (CeA) can increase the anxiety levels of rats, while the inhibition of corticosterone synthesis reduces anxiety-related behaviors (24). Furthermore, CORT acts via glucocorticoid receptors to exacerbate pain in mice with a spared nerve injury (SNI) after exposure to restraint stress (25). Kohda K et al. (26) conducted a study in rats and reported that SPS exposure inhibited the hypothalamo- pituitary- adrenal (HPA) axis and increased GR expression in the hippocampus. Glucocorticoid receptors are known to also be located in spinal cord dorsal horn neurons, and the activation of GRs regulates pain states and the stress response (27). Alterations in spinal circuitry contribute to the exacerbation and maintenance of the pain states when acute pain trans-
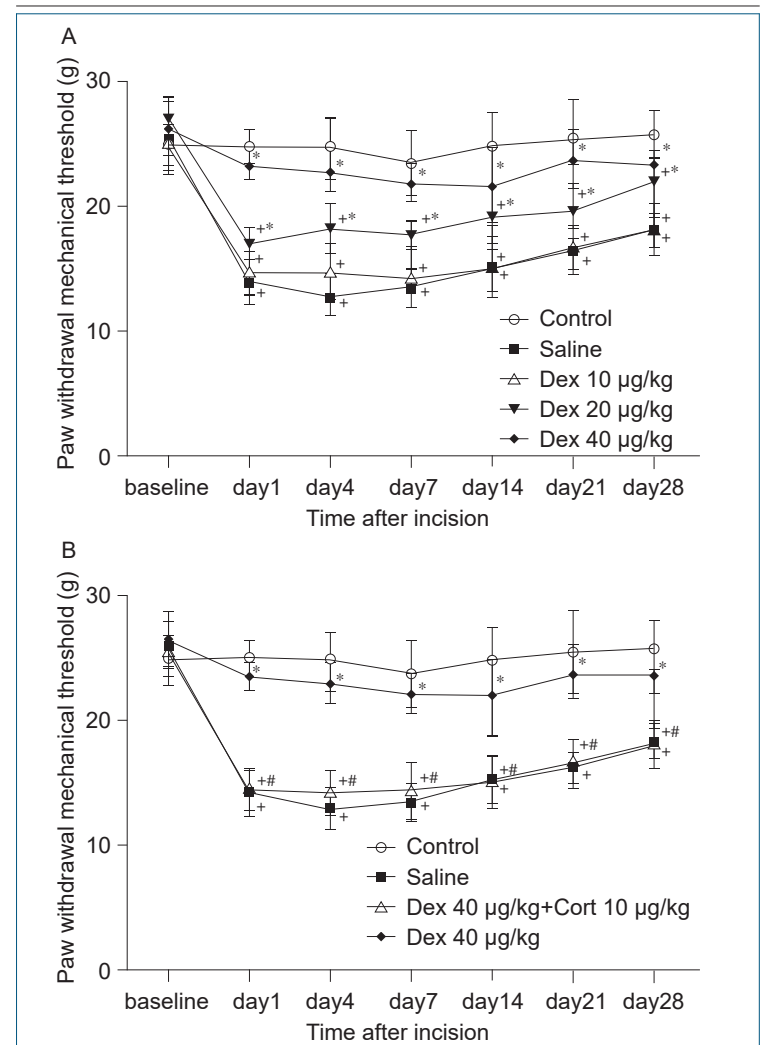

Figure 3. Treatment of Dexmedetomidine Suppressed SPS- Induced Persistent Pain.
A. Intraperitoneal administration of 10 µg/kg dexmedetomidine did not significantly change PWMT from the pre-injection level. However, injection of dexmedetomidine at 20 µg/kg and 40 µg/kg increased PWMT, compared to the pre- injection level, and the effect of the 40 µg/kg dose was more obvious (n = 8 rats for each group). +P<0.05 compared with group C, *P<0.05 compared with saline group; B. Pretreatment with corticosterone blocked the pain inhibition provided by dexmedetomidine. PWMT was measured before the SPS procedure as a baseline level and at 1, 7, 14, 21 and 28 days after plantar incision (n=8 rats for each group). +P<0.05 compared with group C, *P<0.05 compared with saline group and #P<0.05 compared with Dex 40 µg/kg + Cort 10 µg/kg group.

form to persistent pain (28, 29). Therefore, we tested the expression of GR in the spinal cord to study the mechanisms underlying the interactions between anxiety and pain. The levels of circulating corticosterone and spinal cord GR ex-
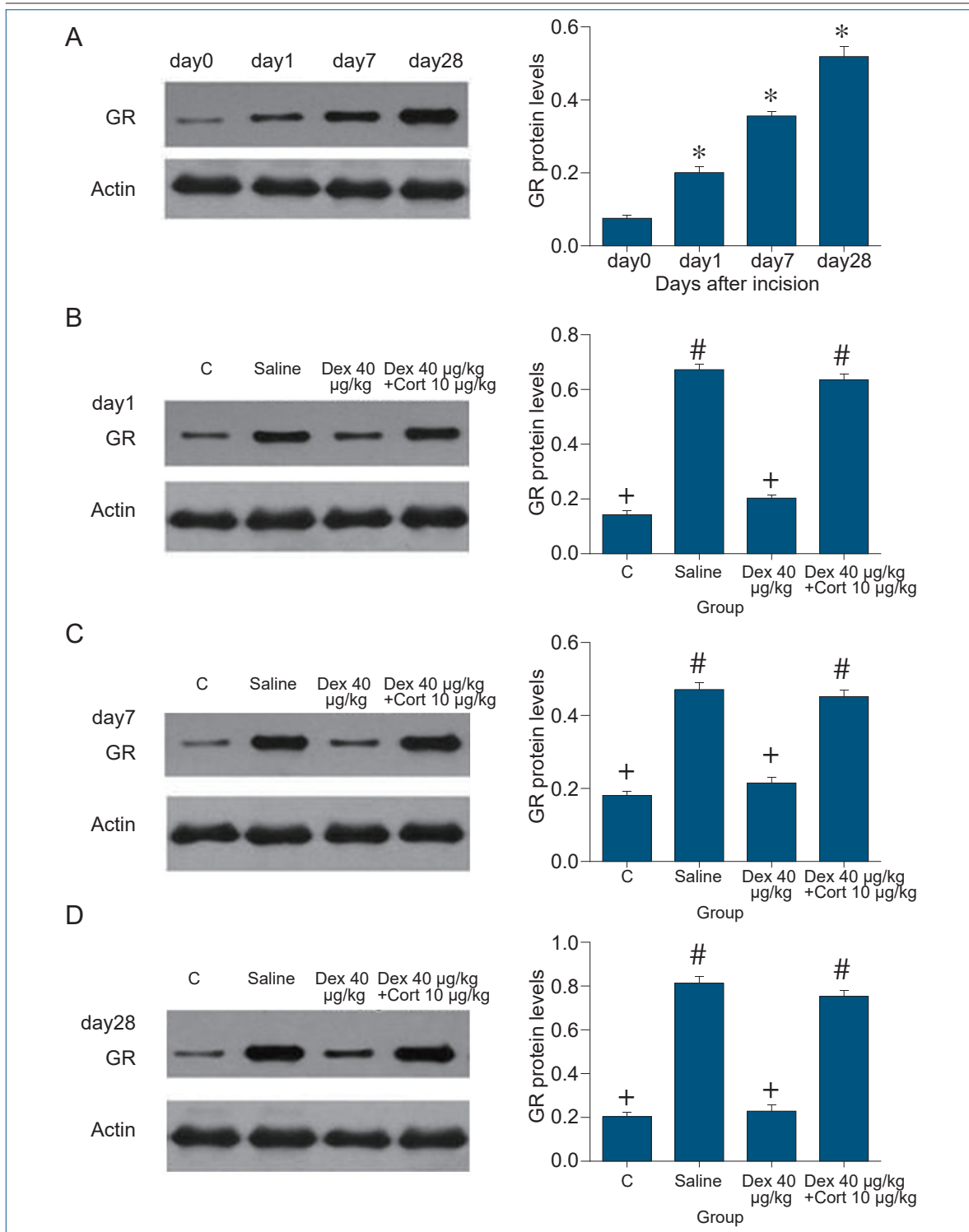
Figure 4. Effect of Dexmedetomidine on the GR Protein Expression in the Spinal Cord (n=3).
The spinal cord dorsal horn of the L4-L5 segment was collected at 1, 7, and 28 days after the incisional opera-
tion. A. GR protein expression was significantly increased in the 'SPS-plus-incision' group compared to the con-
trol group; B-D. At 1, 7, and 28 days after paw incision, dexmedetomidine inhibited the increase in GR expres-
sion observed in the untreated rats, and that effect was blunted by corticosterone. β-actin served as a loading
control. This figure presents the statistical analysis of the relative densities of protein bands observed by Western
blot among the four groups. *P<0.05 compared with day 0, #P<0.05 compared with group C, +P<0.05 compared
with group AI0.

pression increased significantly after exposure to SPS, with a time course that paralleled that of the development of exacerbated and prolonged pain. Furthermore, treatment with the corticosterone synthesis inhibitor metyrapone inhibited the persistent pain induced by SPS. Therefore, this study showed that glucocorticoids are involved in anxiety-induced pain. Microinjections of the α2-adrenoceptor agonist clonidine into the ventrorostral region of the locus coeruleus (LC) inhibited glucocorticoid release (30). Because dexmedetomidine is also a highly selective α2-adrenoceptor agonist, our results showed that the administration of dexmedetomidine inhibited the increase in GR expression in the spinal cord observed in response to SPS and plantar incision. Dexmedetomidine is a α2-adrenergic receptor agonist that has sedative and analgesic properties (31, 32). The intranasal administration of dexmedetomidine before anesthesia has been reported to reduce presurgical anxiety (33). In our study, the administration of dexmedetomidine 30 minutes before surgical incision appeared to attenuate anxiety-like behaviors and persistent pain. The 10 μg/kg dose of dexmedetomidine was ineffective at reducing incisional pain, but the 20 and 40 μg/kg doses ameliorated SPS-induced hyperalgesia. The pain relief effect of the 40 μg/kg dose was greater than that of the 20 μg/kg dose, which suggests that the 40 μg/kg dose may be more beneficial and more suitable for the inhibition of anxiety-induced persistent pain.Thus, dexmedetomidine inhibited anxiety-induced persistent pain in a dose-dependent manner, and this pain inhibition lasted approximately 28 days. Because the half-life of dexmedetomidine is only 2 hours, the sedative effect of dexmedetomidine cannot account for the rats' behaviors because the behavioral tests were performed over a 28-day follow-up period after the dexmedetomidine injection. In this study, dexmedetomidine was administered after, not before, SPS exposure; this timing more closely reflects the clinical situation, giving our results more potential clinical applicability. Furthermore, pretreatment with corticosterone abolished the pain inhibition effect of dexmedetomidine.

Glucocorticoids are clearly involved in anxiety-induced sensory hypersensitivity in the spinal region. Therefore, glucocorticoids may be the key to elucidating the mechanisms underlying the analgesic effect of dexmedetomidine. A great deal of clinical and preclinical results have demonstrated that the nature, duration and intensity of a stressor potently influence pain state (34). Recently, animal studies regarding the effect of psychosocial stress on pain have been conducted. Luis Quintero showed that forced swimming enhanced formalin-induced hyperalgesia in rats (35). Changsheng Li proposed that social defeat stress induces the transition from acute to chronic plantar incision pain (29). Different spinal and supraspinal regions can be influenced by stress; thus, alterations to neurotransmitters within these regions play an important role in stress-pain interactions (36). For example, multiple studies have shown that the cortical structures (37), amygdala (38), periaqueductal grey (39), rostral ventromedial Medulla (28) and spinal cord dorsal horn mediate the process of stress-induced hyperalgesia. Alterations in spinal circuitry have been shown to be responsible for the maintenance and exacerbation of pain. Therefore, we can speculate that anxiety affects the alteration neurochemical reactions at the spinal cord level, which changes spinal cord plasticity, resulting in paw incision, inducing the prolonged and exacerbated pain. Our data showed that GR expression in the spinal cord increased from 1 to 28 days after paw incision and that dexmedetomidine significantly blocked that increase. Our results indicate that the spinal cord is involved in the mediation of anxiety-induced persistent pain.

The precise mechanisms underlying the role of glucocorticoids in anxiety-induced persistent pain remain unclear; there may be multiple underlying mechanisms. Hormones regulate hippocampal excitatory transmission and synaptic plasticity. For example, glucocorticoids promote an increase in the expression of GluA2-containing AMPARs on the synaptic membrane after stress (40). Furthermore, stress and corticosterone both increase extracellular glutamate, and stress-induced activation of GR leads to subsequent NMDA receptor activation (41). However, the stress-induced increase in the circulating levels of corticosterone inhibits GABA release, which, subsequently, causes stress-induced persistent pain (42). These data indicate that these

pathways may converge to cause anxiety-induced persistent pain. Therefore, more work is needed to fully understand the mechanisms of the involvement of glucocorticoids in anxiety-induced persistent pain.

In conclusion, we confirmed that SPS combined with paw incision in rats is a suitable animal model for examining the exaggerated and prolonged pain observed in the presence of high levels of anxiety. Furthermore, glucocorticoids contribute to the development of presurgical

anxiety-induced persistent postsurgical pain. Finally, dexmedetomidine can alleviate anxiety-induced persistent postsurgical pain via decreasing the expression of GR. Therefore, our study indicates that dexmedetomidine is a promising treatment for this clinical condition.

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