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Antinociceptive Effects of Melatonin in a Rat Model of Post-Inflammatory Visceral Hyperalgesia: A Centrally Mediated Process

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Abstract

Previous reports suggest that melatonin may play an important role in visceral nociception and neurogenic inflammation. We aimed to examine the role melatonin on visceral hypersensitivity and to explore the site of action using a rat model of post-inflammatory visceral hyperalgesia. In all rats, a baseline visceromotor response (VMR) to graded colorectal distension (CRD; 10–60 mmHg) was recorded prior and one week following trinitrobenzenesulfonic acid (TNBS) induced colonic inflammation. Melatonin (30, 45 or 60 mg/kg, ip) was given 20 minutes before testing the VMR in naïve and TNBS-treated rats. Extracellular single-unit recordings were made from CRD-sensitive pelvic nerve afferent (PNA) fibers and lumbosacral (LS) spinal neurons in TNBS-treated animals. The effect of melatonin (60 mg/kg) was examined on responses of PNAs and spinal neurons to graded CRD. In separate experiments, luzindole (non-specific MT₁/MT₂ receptor antagonist) or naltrexone (non-specific opioid receptor antagonist) was injected prior to melatonin. Following TNBS, there was a significant increase in the VMR to CRD compared to baseline. This increase was attenuated by melatonin (60 mg/kg) at pressures >20 mmHg. The same dose of melatonin had no effect on the VMR in naïve animals. In TNBS-treated rats, melatonin significantly attenuated the responses of CRD-sensitive spinal neurons to CRD, but had no effect in spinal transected rats or PNA fibers. Both luzindole and naltrexone blocked melatonin's effect on the VMR and LS spinal neurons. Results indicate melatonin's antinociceptive effects are not via a peripheral site of action but rather a supraspinal process linked to the central opioidergic system.

Keywords

Melatonin; colorectal distension; visceral hyperalgesia; MT receptors

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Introduction

Melatonin, (N-acetyl-5-methoxytryptamine) a derivative of serotonin (5-HT), is a neurohormone that is primarily produced by the pineal gland. It has been implicated in the control of the wake-sleep cycle [18] and also motility of the gastrointestinal tract [6]. In fact, a large source of endogenous melatonin comes from the gastrointestinal tract [4]. Recently, evidence has been mounting to support the role of melatonin in pain regulation [7,29]. Several studies have suggested that receptors for melatonin (MT₁ and MT₂) are expressed throughout the nervous system including the thalamus, hypothalamus, anterior pituitary, and in the dorsal horn of the spinal cord where they are believed to be involved in nociceptive transmission [20]. While the MT₁ and MT₂ receptors appear to play an important role in sleep, their interactions with other systems including the serotonergic, dopaminergic, adrenergic or opioidergic pathways have been proposed as potential mechanisms involved in mediating the antinociceptive effects of melatonin [13].

Clinical studies in humans have also documented the benefit of melatonin in improving pain in conditions such as irritable bowel syndrome (IBS), fibromyalgia and migraine headaches [21,12,24,27]. In fact, melatonin has been shown to be superior to placebo in decreasing abdominal pain and extracolonic symptoms in IBS patients as well as improving symptoms of functional dyspepsia [24]. While it has been suggested that melatonin has anti-inflammatory properties, it is unlikely that the antinociceptive effect is through this mechanism alone [5].

To date, the precise mechanisms through which melatonin attenuates hypersensitivity is not well understood. Multiple studies have demonstrated the antinociceptive effect of melatonin in animal models of acute, inflammatory and neuropathic pain [29,7,1]. While some studies have shown that melatonin significantly decreases spinal dorsal horn neuron firing following stimulation of C fiber neurons [11], others have shown that it inhibits voltage-activated calcium channels in cultured rat dorsal root ganglion (DRG) cells, suggesting a peripheral effect [3]. A significant number of recent studies have proposed a potential link to the central opioidergic system as a mechanism underlying the antinociceptive effect of melatonin [29,14]. To our knowledge, no studies have attempted to investigate the effect and potential mechanism of melatonin in a model of post-inflammatory visceral hyperalgesia.

We aimed to investigate the site of action and the mechanism underlying the antinociceptive properties of melatonin in a rat model of post-inflammatory hyperalgesia. Our systematic approach evaluated, 1) visceral analgesic property of melatonin in awake, naïve versus inflamed rats, 2) effects of melatonin on the mechanotransduction properties of CRD-sensitive pelvic nerve afferent (PNA) fibers and lumbosacral (LS) spinal neurons, and 3) effect of melatonin in the presence of the opioid receptor antagonist naltrexone or the MT₁/MT₂ receptor antagonist luzindole.

Methods

Animals

The study was carried out using male Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) with an average weight of 400 g (range: 350-450 g). Rats were kept in controlled conditions with a 12 hour light/dark schedule and had access to both food and water ad libitum. Twenty-four hours before surgery, the animals were placed in a wire-bottom cage and access to food, but not water, was denied in order to empty the colon. All experiments were performed according to the approved guidelines of the institutional Animal care and Use committee at the Medical College of Wisconsin and The International Association for the Study of Pain (IASP).

Drugs

Melatonin and naltrexone were purchased from Sigma Aldrich (Norwich, NY) and dissolved in propylene glycol and water, respectively. Luzindole was obtained from Tocris Bioscience (Ellisville, MO) and was dissolved in 100% dimethyl sulfoxide (DMSO).

Recording of visceromotor response (VMR)

Surgical procedure—Rats were initially anesthetized by injecting pentobarbital sodium (45-50 mg/kg, ip) (Ovation Pharmaceuticals Inc. Brown Deer, IL, USA). Teflon coated electrodes (Cooner Wire, part # A5631, Chatsworth, CA, USA) were implanted in the external oblique muscle for electromyography (EMG) recordings. The electrodes were externalized dorsally near the neck and secured in place with silastic tubing.

VMR Recordings—No less than 72 hours after surgery, rats were placed inside the plexiglass restraining tubes for two hours on three consecutive days in order to acclimatize them to experimental conditions. On the day of VMR recordings (6-7 days after electrode implantation), rats were placed in the restraining tube and a highly compliant, flaccid latex balloon (6 cm long and 3.5 cm OD) coated with non-reactive bacteriostatic lubricant (Surgilube, E. Fougera, Melville, USA) was inserted into the descending colon and taped to the tail. Rats were allowed to rest inside the tube for at least 30 minutes before testing the VMR to colorectal distension (CRD). The EMG signal was amplified using the A-M System amplifier (model 1700, Carlsborg, WA, USA). A stimulus-response function (SRF) to graded CRD (10, 20, 30, 40, 60 mmHg) was recorded. The duration of distension was 30s with a 180s inter-stimulus interval between the distension. Data were recorded real-time using the Spike 4/CED 1401 data acquisition program (CED 1401; Cambridge Electronic Design, Cambridge, UK). Following a baseline SRF, rats were lightly anesthetized with pentobarbital sodium (10-20 mg/kg, ip) and 0.5 ml of 50% tri-nitrobenzenesulfonic acid (TNBS, dissolved in ethanol) was slowly injected into the descending colon using a 16 gauge gavage needle. Rats were then allowed to recover for 7 days prior to repeating the VMR.

Electrophysiology

PNA Recordings—Following TNBS treatment, rats were allowed to recover for 7 days prior to performing any electrophysiology experiments. For PNA fiber recordings, a 3 - 4 cm long incision was made in the lower abdomen after anesthesia and the prostate lobe was reflected laterally to access the major pelvic ganglion (MPG), pelvic and hypogastric nerves. The pelvic nerve was isolated from the surrounding fatty tissues and a pair of teflon-coated stainless wires were placed around it proximal to the MPG to deliver electrical stimulation to pelvic nerve in order to confirm that recording were made from the dorsal sacral root. Electrodes were secured in place by draping a piece of peritoneal fat tissue around it and the abdomen was closed with silk sutures. A laminectomy was performed to expose the lumbo-sacral (T₁₃-S₂) spinal cord and the rat was stabilized by clamping the thoracic vertebra and hip joint. The dorsal skin was opened, reflected laterally and tied to make a pool for mineral oil. The dura was carefully removed and the spinal cord was covered with warm (37°C) mineral oil. Recordings were made from the distal cut end of the central processes of the sacral S1 dorsal root as previously described [25]. PNA projections in the S1 dorsal root were identified by recording the evoked response to electrical stimulation (5-10 mv, 0.5ms square wave pulse). The identified fiber was the tested to CRD (30 mmHg) and once confirmed as a CRD-sensitive fiber, a SRF was constructed to graded CRD (10-60 mmHg, 30 s, 3 min interstimulus interval). Action potentials were processed through a window discriminator and the frequency of impulses was counted (1s binwidth) on-line using the spike2/CED 1401 data acquisition program. Peri-stimulus time histograms (PSTH, 1 s binwidth), colonic distending pressures, and blood pressure were displayed on-line. The CRD-sensitive PNA or spinal neurons were identified by response to

CRD (30 mmHg, 2-3s). The balloon catheter was connected to a computer-controlled barostatic distension device.

Recording LS spinal neurons—Rats were anesthetized initially with sodium pentobarbital (40-45 mg/kg ip) and maintained with supplemental doses of 5 mg/kg/hr. The trachea was cannulated to mechanically ventilate the rat. The left common carotid artery was cannulated for recording blood pressure and the femoral vein was catheterized for injection of anesthetic and muscle relaxant. The rat was paralyzed with gallamine triethiodide (1 mg/kg, iv) and ventilated with room air (55-60 strokes/min and 3-4 ml stroke volume). Additional doses of gallamine triethiodide (0.5 mg/kg/h) were given to maintain paralysis during the course of the experiment. A PE-50 catheter was placed in the abdominal cavity in close proximity of the descending colon to deliver melatonin intraperitoneally (ip).

For spinal transection, a laminectomy was performed to expose the cervical (C1–C2) spinal cord. The dura membrane was gently removed and 10–15 μ l of 2% lidocaine was applied to the dorsal surface of the exposed spinal cord. After 10 minutes, the spinal cord was completely transected by using a scalpel blade. The transected area was covered with a small piece of gelfoam soaked in warm saline. Animals exhibited a vasodepressor response immediately after spinal transection that recovered in 15–30 min. All spinal recordings were made at least one hour following spinal transection.

The LS spinal cord was exposed by laminectomy (T13-S2) and the rat was suspended from the thoracic vertebral and ischial spinal clamps. The dorsal skin was reflected laterally and tied to make a pool for mineral oil. The dura was carefully removed and a piece of gelfoam (Ferrosan, Soeborg, Denmark) was placed on the spinal cord. The pool was fully covered with 1.75% agar in saline and after hardening of the agar a small window was cut to expose the spinal cord. Stainless steel microelectrodes (8-10 M Ω , FHC, Bowdoinham, ME) were used for extracellular recording. The placement of the electrode was 0.1-0.5mm lateral from the spinal midline and 0.6-1.3mm ventral from the dorsal surface. The SRF was constructed following the method used for PNA recordings.

The action potentials were amplified through a low-noise AC differential amplifier (model 3000; A-M Systems) and continuously monitored and displayed on an oscilloscope. The spinal neurons were classified based on their response characteristics to mechanical distension as either short latency abrupt (SLA) or short latency sustained (SLS) as previously described [15]. Post experiments, data were analyzed using the Wave-Mark analysis method of the Spike 4 software (CED, Cambridge, UK) to distinguish individual action potentials.

Experimental Protocol

Behavioral study—The effect of melatonin was tested on the VMR to CRD of non-inflamed, naïve and TNBS-treated rats. The drug was given intraperitoneally (ip) in all experiments, and a SRF to graded CRD (10-60 mmHg) was recorded before and 20 minutes following melatonin injection. In order to determine the effective antinociceptive dose, melatonin was given at three different doses, 30, 45 and 60 mg/kg. To explore the mechanism of action of the drug, it was tested in the presence of two antagonists administered 10 minutes prior to injection of melatonin; 1) luzindole (5 mg/kg, ip), a non-specific MT₁/MT₂ antagonist or 2) naltrexone (5 mg/kg, sc), a non-selective opioid receptor antagonist. Additional VMR recordings were performed in TNBS-treated rats to test the effect of propylene glycol (the solvent for melatonin), luzindole or naltrexone.

Electrophysiology Study—A baseline SRF to graded CRD of PNAs was constructed in TNBS-treated rats and repeated 20 minutes after injection of melatonin (60 mg/kg, ip). Similarly, in a separate set of animals, CRD-sensitive LS spinal neurons from TNBS-treated

rats were identified and SRFs to graded CRD were constructed before and 20 minutes after melatonin injection (60 mg/kg, ip). In order to test the receptor specific action of melatonin and its association with the opioidergic system, experiments were performed in animals pre-treated with either luzindole (5 mg/kg, ip) or naltrexone (5 mg/kg, iv). These antagonists were administered 10 minutes prior to injection of melatonin.

To investigate the central site of action of melatonin, in a separate set of experiments, responses of spinal neurons to CRD were recorded after cervical (C1-C2) spinal transection to eliminate the supraspinal influence and the effect of melatonin (60 mg/kg, ip) was tested.

Data analysis

Statistical analysis was performed using SigmaStat (V2.03, SPSS Inc, Chicago, IL). A SRF to graded CRD was constructed to test the intensity dependant increase in EMG activity. The area under the curve was measured from the EMG recordings and taken as a percent of maximum response from baseline (60 mmHg). Therefore, each animal served as its own control.

For analysis of PNA fibers and LS spinal neuron's responses to CRD, the total number of action potentials over 60 second resting period prior to colon distension and during the distension period (30 seconds) was counted and represented as impulses/sec. To measure the actual changes in response of the neurons to CRD, the mean firing frequency during the resting period was subtracted from the mean firing frequency during colon distension. Neuronal firing before and after treatments was analyzed as a percent of maximum response from baseline (60 mmHg). Statistical analysis was performed using two-way or repeated-measures ANOVA and by Student-Newman-Keuls. All results are expressed as mean $\pm$ SEM. Values with $p < 0.05$ were considered to be significant.

Results

VMR

In naïve rats, melatonin (30 or 60 mg/kg, ip) had no effect on the VMR at all CRD pressures tested ($p > 0.05$, Fig. 1A and B). Seven days following intracolonic TNBS, rats developed a significant visceral hyperalgesia compared to baseline responses. This hypersensitivity was evident at CRD pressures > 30 mmHg ($p < 0.05$) (Fig. 2A-D). At doses of 30 or 45 mg/kg, melatonin had no effect on the VMR of TNBS-treated animals (Fig. 2A and B, respectively). However, at a higher dose (60 mg/kg), melatonin significantly decreased the VMR at CRD pressures ≥ 20 mmHg compared to the post-TNBS response (Fig. 2C). The vehicle for melatonin, propylene glycol, had no effect on the VMR of TNBS-treated rats (Fig. 2D). Figure 3 shows an example of a typical EMG response of one rat before and after TNBS and following melatonin. As a result of these findings, all subsequent experiments were performed using the dose of 60 mg/kg of melatonin.

In a separate set of experiments, TNBS-treated rats were treated with luzindole, prior to melatonin administration. The inhibitory effect of melatonin on the VMR was prevented by luzindole, suggesting that melatonin produces analgesia by acting directly on melatonin receptors (Fig. 4A). Similarly, pre-treatment with naltrexone also blocked the antinociceptive effect of melatonin in TNBS-treated rats (Fig. 4B). In order to rule out the possibility of these antagonists having pronociceptive effects, either luzindole or naltrexone were given alone prior to testing the VMR. Neither antagonist had any effect on the VMR response (Fig. 4C and D).

Electrophysiology

Effect of melatonin on colonic PNA fibers—In order to examine the peripheral site of action of melatonin, we tested its effect on the responses of low threshold (threshold ≤ 10

mmHg), mechanosensitive PNA fibers to graded CRD. Melatonin did not attenuate the spontaneous firing (0.86 ± 0.24 mean imp/sec, pre-melatonin vs 0.198 ± 0.05 post-melatonin) or the mechanotransduction of PNA fibers in TNBS treated animals (Fig. 5A). Figure 5B shows an example of a typical response characteristic of a PNA fiber before and after melatonin (60 mg/kg) injection.

Effect of melatonin on CRD-sensitive LS spinal neurons—Similar to the VMR experiments, melatonin had no effect on CRD-sensitive LS spinal neurons in the naïve animal (Fig. 6A). However, in TNBS-treated rats there was a significant reduction of firing following melatonin injection at CRD pressures ≥ 20 mmHg (Fig. 6B). Figure 6C illustrates responses of a typical CRD-sensitive LS spinal neuron from a TNBS treated rat before and after melatonin injection.

In TNBS-treated rats, pre-treatment with luzindole blocked the effect of melatonin on the response of the neurons to mechanical distension (Fig. 7A). An example of single neuron response to luzindole plus melatonin is shown in figure 7B. Similarly, naltrexone given 10 minutes prior to melatonin blocked the inhibitory effect of melatonin (Fig. 8A). An example of a CRD-sensitive spinal neuron's response to graded CRD following naltrexone and melatonin injections is shown in figure 8B. The population of spinal neurons from the intact spinal cord along with the average spontaneous firing and response to the maximum distension of 60 mmHg is described in table 1.

Unlike the effect observed in intact animals, melatonin did not inhibit the neuronal responses to CRD in spinal transected animals (Fig. 9). This suggests that melatonin produces the effect on spinal neurons by acting at a supra-spinal site.

Discussion

The present study involved behavioral and electrophysiology experiments from peripheral and spinal cord neurons to investigate the effect and potential mechanism involving the antinociceptive effects of melatonin in a rat model of visceral hyperalgesia. While the antinociceptive effects of melatonin have been shown in several models of chemical and thermal nociception in mice and rats [8,30,17], to our knowledge, this is the first report of its effect on post-inflammatory visceral hyperalgesia. The results from this study show that melatonin does indeed attenuate post-inflammatory hypersensitivity. Our results also indicate that it is not a peripheral site of action, but rather a supraspinal process linked to the central opiodergic system.

The receptors for melatonin (MT_1 and MT_2) are expressed throughout the gastrointestinal tract and in the central nervous system [4]. A recent study found these receptors to be abundantly expressed in spinal cord dorsal horn cells [19]. In addition, spinal neuron synaptic potentiation has been shown to be inhibited by melatonin [16]. Taken together, these data suggest that the antinociceptive effect of melatonin may result from a direct effect on spinal cord neurons. The current study however, contradicts this hypothesis. While the recordings made from spinal cord neurons clearly demonstrate a decrease in response to CRD following melatonin injection, the same was not evident in spinal transected rats. In other words, melatonin fails to decrease the firing of spinal neurons if the supraspinal influence is removed, suggesting a minimal or no effect of melatonin directly on dorsal horn neurons.

Since melatonin has high lipid solubility and can easily penetrate the blood-brain barrier, it has been suggested that the hormone mainly acts at supraspinal sites [30]. Interestingly, it has been shown in mice, that melatonin treatment (1-100 mg/kg, ip) does not cause any change in locomotor activity in the open field test compared to vehicle [13]. Evidence in favor of a

supraspinal site of action is mounting. A recent study using tail flick latency documented that the antinociceptive effects of melatonin were counteracted by intercisternal administration of naloxone [29]. Similar results were demonstrated in a separate study with luzindole given intracerebroventricular (icv) [30]. These results, combined with the recordings from spinalized rats in the present study, provide compelling support for the supraspinal site of action of melatonin. Additionally, we have documented that melatonin does not have a direct effect on primary sensory neurons, since it failed to alter their spontaneous firing and response to CRD in sensitized rats.

Endogenous opioid peptides such as endorphins and dynorphins can produce analgesia by interacting with delta, kappa and mu receptors in the spinal cord and supraspinal areas. However, it appears unlikely that melatonin induced analgesia is mediated through direct binding of melatonin to opioid receptors since neither melatonin agonists nor antagonist bind to opioid receptors [26]. In the present study, both naltrexone and luzindole individually reversed the antinociceptive effects of melatonin in the behavioral studies and blocked the inhibitory effect of melatonin on spinal cord neurons. These results strongly suggest that the mechanism underlying the melatonin induced anti-nociception is linked to the central opioidergic system. In fact, previous studies using different models of pain also have suggested this link. Yu et al. (2000) found that exogenously administered melatonin increases the release of endogenous opioids, especially β -endorphin from the rat periaqueductal gray area and increases somatic pain thresholds [28,30]. Similar to the findings in the current study, these investigators also found that luzindole was able to block the antinociceptive effect of melatonin. The fact that both luzindole and naltrexone block the effect of melatonin provides strong evidence that both MT_1/MT_2 and opioid receptors are involved in the antinociceptive effect. It is likely that naltrexone indirectly antagonizes melatonin by blocking the binding of endogenous opioids to their receptors.

Another interesting finding in the present study is the lack of effect of melatonin on VMR and CRD-sensitive spinal neurons in naïve animals. Our data clearly demonstrate that melatonin administered systemically, elicits a significant decrease in the VMR to CRD only in TNBS-sensitized rats, but not in naïve, non-sensitized rats. Thus, it appears that melatonin works as an “anti-hyperalgesic” as previously described in other inflammatory and neuropathic pain models [1,2] Further studies are necessary to determine the exact pathway involved, however, it can be speculated that the pathway may also involve other channels known to mediate excitatory synaptic transmission such as NMDA and/or 5-HT receptors that have been previously linked to melatonin [14,9]. Additionally, other studies have suggested that melatonin possesses immunomodulatory and anti-inflammatory effects that may impart protective effects against gastrointestinal inflammation. However, the acute effects of melatonin in both the behavioral and electrophysiology studies are unlikely the result of an anti-inflammatory effect that could account for the decrease in neuronal firing and/or pain sensitivity observed in the current study [22,23,10].

In summary, our findings extend previous literature regarding the antinociceptive effects of melatonin and provide additional insight into the potential mechanism involved in a rat model of post-inflammatory, visceral hyperalgesia. We have shown that melatonin significantly decreased the VMR to CRD in awake animals and attenuates the firing of sensitized spinal neurons. Our data clearly shows that melatonin has no effect on primary sensory afferents but rather exerts its effect in supraspinal areas via the central opioid system.

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Figure 1.

The mean VMR to graded CRD before and after melatonin in naïve rats. Melatonin, 30 mg/kg (A) or 60 mg/kg (B) had no effect on the VMR of naïve rats.

Figure 2.

The mean VMR to graded CRD before and after melatonin in rats one week after intracolonic TNBS. Rats exhibited significant hyperalgesia following TNBS that was not affected by melatonin 30 mg/kg (A) or 45 mg/kg (B). At the higher dose 60 mg/kg, there was a significantly decreased in response to CRD pressures >20 mmHg (C). The vehicle for melatonin (propylene glycol) had no effect on the VMR (D) * $p < 0.05$ vs pre-TNBS, # $p < 0.05$ vs post-TNBS.

Figure 3.

VMR represented as EMG activity to graded CRD (20, 30, 40 mmHg) from naïve, pre-TNBS (A), post-TNBS (B) and post-TNBS following melatonin (60 mg/kg) (C). The VMR following TNBS was higher than pre-TNBS. Melatonin decreased the EMG response in post-TNBS rats.

Figure 4.

The mean VMR to graded CRD in TNBS-treated rats. Melatonin has no effect on the VMR in the presence of luzindole (5 mg/kg, ip) (A). Melatonin has no effect on the VMR in the presence of naltrexone (5 mg/kg, sc) (B). Luzindole (C) or naltrexone (D) alone had no effect on VMR of TNBS-treated rats. * $P < 0.05$ vs pre-TNBS.

Figure 5.

The mean stimulus-response function of pelvic nerve afferents from TNBS-treated rats before and after melatonin shows that melatonin had no effect on the afferent's responses to graded CRD (A). Example of responses of a CRD-sensitive pelvic afferents at different intensities of distension before and after melatonin is shown in (B). In all panels, the top trace shows the response to CRD represented as a frequency histogram (1 s bin width), the middle trace is the neuron action potential and the bottom is the distension pressure.

Figure 6.

The mean SRF of LS spinal neurons in naïve and TNBS-treated rats before and after melatonin. Melatonin had no effect on spinal neurons in naïve rats (A). The response of spinal neurons to CRD in TNBS-treated rats was significantly decreased by melatonin at pressures >10 mmHg (B). Example of the typical response of a CRD-sensitive LS neuron from a TNBS-treated rat is shown in (C). In all panels, the top trace shows the response to CRD represented as a frequency histogram (1 s bin width), the middle trace is the neuron action potential and the bottom is the distension pressure. * $P < 0.05$ vs TNBS.

Figure 7.

The mean SRFs of LS spinal neuron showed that melatonin had not effect on spinal neurons in the presence of luzindole (5 mg/kg) (A). A typical example of a CRD- sensitive LS spinal neuron from a TNBS- treated rat and following administration of melatonin and luzindole is shown in (B).

Figure 8.

The mean SRFs of LS spinal neuron showed that melatonin had not effect on spinal neurons in the presence of naltrexone (5 mg/kg) (A). A typical example of a CRD- sensitive LS spinal neuron from a TNBS- treated rat and following administration of melatonin and naltrexone is shown in (B).

Figure 9.

The mean SRF of CRD-sensitive LS spinal neurons in TNBS-treated, spinalized (C₁-C₂) rats before and after melatonin (60 mg/kg ip). Melatonin had no effect on the response of spinal neurons to CRD in spinalized rats at all distensions tested.

Table 1

Response characteristics of lumbrosacral spinal neurons from naïve and TNBS-treated animals

Types of Neurons	Number of Neurons	Spontaneous firing	Firing at 60 mmHg
SL-Abrupt - Naïve	4	0.63 ± 0.61	7.87 ± 2.87
SL-Sustained - Naïve	2	0.74 ± 0.76	4.36 ± 0.62
SL-Abrupt - TNBS	11	0.87 ± 0.53	13.12 ± 2.17
SL-Sustained -TNBS	6	1.11 ± 0.48	10.29 ± 1.49