Neuropharmacology and analgesia

Effect of dexmedetomidine and cold stress in a rat model of neuropathic pain: Role of interleukin-6 and tumor necrosis factor-α

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ABSTRACT

Dexmedetomidine (Dex) is a novel Alpha 2-adrenoceptor agonist. It decreases sympathetic tone and attenuates the stress responses to anesthesia and surgery. People exposed to cold suffer unpleasant thermal pain, which is experienced as stress and causes the release of noradrenaline from the sympathetic terminals. The present study investigated the effects of cold stress and dexmedetomidine on chronic constriction injury (CCI) model of the sciatic nerve in rats. Sixty four male Wistar rats were divided into seven groups of eight rats each: repeated cold stress (RCS) group, sham RCS group, CCI group, sham CCI group, Dex-treated group received a single dose of Dex (5 μg/kg), CCI + Dex group, CCI + RCS group, Interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) levels in the serum were measured by enzyme-linked immunosorbent assay. The mean body weight of CCI, RCS, CCI + RCS, CCI + Dex and RCS + Dex groups decreased significantly compared with pre-values. Dexmedetomidine and CCI caused significant changes of the systolic, diastolic and mean blood pressure. Both RCS and CCI groups showed significant decreased of reaction time in the hot plate test. The RCS and CCI groups demonstrated a significant mechanical hyperalgesia, while pain threshold was increased in the RCS + Dex group. A significant decrease of serum IL-6 and TNF-α was demonstrated in CCI + RCS and CCI + Dex groups. The therapeutic effectiveness of dexmedetomidine in neuropathic pain may be through inhibition of proinflammatory cytokines, primarily IL-6 and TNF-α. Moreover, cold stress may result in increased resistance to neuropathic pain.

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1. Introduction

Neuropathic pain, resulting from lesions or diseases of the central or peripheral nervous system, affects 7–8% of the population (Torrance et al., 2013). The abnormal sensory processing in either the peripheral or central nervous system is characterized by spontaneous pain, increased responsiveness to painful stimuli (hyperalgesia), and pain perceived in response to normally non-noxious stimuli (alldynia) (Sandkühler, 2009). Nerve damage can trigger molecular changes such as up-regulation of sodium channels and receptors in nociceptive neurons that become abnormally sensitive and develop pathological spontaneous activity. The hyperactivity in nociceptive neurons consecutively induces secondary hyperexcitability in upstream neurons in the spinal cord and brain. This central sensitization causes input from mechanoreceptive A-fibers to be perceived as mechanical allodynia (Liljencrantz and Olausson, 2014).

Another important example of stressful conditions is repeated cold stress defined as rapid and frequent environmental temperature changes that occurs several times within a day (Tazumi et al., 2005). Under stressful conditions, many behavioral changes, autonomic, hormonal, and immune responses are elicited. Stress can induce changes in cytokine and neurotransmitter levels in the hypothalamus (O’Connor et al., 2000).

Dexmedetomidine (Dex) has been successfully used for attenuating stressful conditions (Tang et al., 2015). It selectively binds to presynaptic α2 adrenergic receptors, resulting in a reduction of norepinephrine release and postsynaptic adrenergic discharge (Giovanitti et al., 2015). Dex is an effective sedative agent and also provides analgesia with minimal influence on the respiratory system. Clinical and experimental studies revealed that Dex significantly decreased the levels of inflammatory cytokines with potential anti-inflammatory and antioxidant effects (Jang et al., 2014; Wang et al., 2015; Xianbao et al., 2013; Zhang et al., 2014).

It has been proposed that irrespective of the characteristic of the pain, whether it is sharp, dull, aching, burning, stabbing, numbness, or tingling, all pains arise from inflammation and the
inflammatory response (Omoigui, 2007). The debate is still ongoing regarding the role of both immune and inflammatory mediators in neuropathic pain and stress conditions where neurons and glia may produce a variety of inflammatory cytokines, including tumor necrosis factor- alpha (TNF-α), interleukin-1beta (IL-1β), interleukin-6 (IL-6) and interleukin-10 (IL-10). Cytokines consecutively induce expression of cyclooxygenase, inducible nitric oxide synthase, and substance P, leading to increased nociceptive discharge and thus allodynia and hyperalgesia (Baron, 2009). In general, the pathophysiology and treatment of neuropathic pain are still unsatisfactory. Therefore, our hypotheses are 1) Inflammatory cytokines may play an important role in the pathophysiology of neuropathic pain and cold stress 2) Dex may ameliorate neuropathic pain through inhibition of inflammatory cytokines such as IL-6 and TNF-α. 3) Cold weather or stress enhances sympathetic outflow and hence, may worsen neuropathic pain via an effect on inflammatory cytokines.

2. Materials and methods

2.1. Chemicals and drugs

Drugs were obtained from the following sources: Dexmedetomidine hydrochloride (Precedex®, Hospira, Inc. Lake Forest, IL, USA), Pentobarbital sodium (Sigma Chemical Co., USA). Rat ELISA kits for determination of IL-6 and TNF-α kits were obtained from Koma Biotech Inc. The dose of dexmedetomidine was chosen according to previous studies in which no toxicity or motor impairment was identified (Farghaly et al., 2014; Guneli et al., 2007).

2.2. Animals

Experiments were conducted using sixty four adult male Wistar rats weighing 200–400 g, obtained from the animal house facility, Faculty of Medicine, Assiut University. The animals were housed four per cage under controlled temperature (22 ± 1 °C) except during the period of repeated cold stress (RCS) treatment. All experiments in the present study were conducted according to the Regulations for Ethical Guidelines of the International Association for the Study of Pain (Zimmermann, 2001). The experiments reported here were approved by our institutional ethics committee.

2.3. Experimental protocol

Rats were divided into eight groups (n=8 for each group) as follows:

- 1st group of animals was exposed to repeated cold stress (RCS) at 4 °C every day for 7 days (RCS group),
- 2nd group was kept without any temperature shifts (temperature was kept at 22 ± 1 °C) and served as an unstressed control (Sham RCS group),
- 3rd group had been exposed to chronic constriction injury (CCI) of the sciatic nerve (CCI group),
- 4th group was exposed to an identical dissection in the left paw, except that the sciatic nerve was not ligated (Sham CCI group),
- 5th group received a single intraperitoneal (i.p.) dose of dexmedetomidine hydrochloride (5 μg/kg) (Dex group),
- 6th group received single i.p. dose of dexmedetomidine hydrochloride (5 μg/kg) seven days after ligation of the sciatic nerve (CCI+Dex group),
- 7th group was exposed to cold stress paradigm daily for 7 days immediately on the next day after sciatic nerve ligation (CCI+RCS group),
- 8th group received single i.p. dose of dexmedetomidine hydrochloride (5 μg/kg) seven days after exposure to cold stress paradigm (RCS+Dex group).

2.4. Methods

In the first set of experiments, three groups of rats were employed. The effect of cold stress was studied by placing the rats, while in their metal-mesh cage, in a refrigerator at 4 °C for four hour from 9.00 to 1.00 p.m., and the procedure was repeated every day for 7 days (Kuraishi et al., 1990). Control groups of animals, i.e. Sham RCS and Sham CCI remained undisturbed in their home cage for the same period of time. Every day after the completion of RCS stress, animals were subjected to blood pressure measurements and nociceptive tests.

In the second set of experiments, neuropathic pain model in the form of chronic constriction injury procedure was performed. The CCI procedure was performed as previously described (Bennett and Xie, 1988). Briefly, rats were anesthetized with 50 mg/kg i.p. sodium pentobarbital. The common left sciatic nerve was exposed and a 7–10 mm length of the sciatic nerve, proximal to the sciatic trifurcation was carefully cleared from underlying tissue using blunt dissection. Four ligatures (4.0 braided silk) were tied loosely around it at one mm intervals. The muscle groups approximated and the skin incision was closed with silk suture and was then covered with iodine. In sham CCI animals, an identical dissection was performed in the left paw, except that the sciatic nerve was not ligated. All surgical procedures were carried under strict sterile conditions.

The repeated cold stress procedure was started on the following day after CCI and cold exposure was repeated for 7 consecutive days. Before and eight days after ligation of the sciatic nerve, we determined both blood pressure level and nociceptive sensitivity. Cytokines such as IL-6 and TNF-α were determined both blood pressure level and nociceptive thresholds prior to injection of dexmedetomidine. Thirty minute later, at the peak effect of dexmedetomidine, the same previous parameters were re-tested.

2.5. Nociceptive tests

2.5.1. The hot plate test

To evaluate acute centrally mediated nociceptive signaling, the hot plate test was employed. The surface temperature of the plate (Socrel DS37, UGO Basile, Italy) was maintained at 55 ± 0.5 °C (a temperature at which both A- and C-fibers are activated throughout the experiment). Rats were gently placed; the reaction time of each animal (licking the hind paw of the injured side or jumping at the plate) was measured as the pain response. A standard 40 s cutoff time was used as a maximal effect. Reaction time was determined and the average of three readings taken approximately 5 min apart was calculated (Espejo and Mir, 1993).

2.5.2. The paw pressure (PP) test (Randall-Selitto test)

Nociceptive threshold after sciatic nerve ligation was evaluated using a PP test. The animals were gently restrained with a towel around the trunk to calm them, and treated gently during the experiments and incremental pressure was applied using the available 7200 analgesymeter (Ugo Basile, Italy) onto the dorsal surface of the hind paw which was placed on a small plith under a cone-shaped pusher with a rounded tip. A pedal switch was depressed to exert the force that increases at a constant rate (a certain number of grams per second). The force was monitored by a pointer moving on a linear scale.

When the animal struggles, the pedal was released and the
scale at which the animal felt pain was determined. After each test, the slide was returned to its starting point by lifting it and pushing it to the left. The force was measured on the scale calibrated in 10 g steps by a pointer riveted to the slide. The cutoff pressure was 200 g to avoid damage of the tissue.

The intensity of the pressure that caused an escape reaction was defined as the pain threshold (expressed in grams). Measurements were performed in triplicate at about 90 s intervals and the mean value was taken as the threshold. The PP test was conducted 30 min after Dex administration. Determination was also made prior to CCI and thereafter.

2.5.3. Electronic von Frey test
Assessment of mechanical allodynia was measured in different groups by the use of an electronic von Frey apparatus (Model EVF3, Bioseb, France). Animals were placed in a quiet room in plastic cages with a wire grid floor 30 min before paw stimulation. The electronic von Frey polypropylene tip was applied perpendicularly to the midplantar surface of the hind paw and the intensity of the stimulus was automatically recorded when the paw was reflexly flexed followed by a clear flinch response after paw withdrawal (Chaplan et al., 1994).

2.6. Blood pressure measurements
Before starting the experiment, rats were trained for 7 days in a restrainer to get them acclimatized with the laboratory environment. Baseline measurements of systolic (SBP), diastolic blood pressure (DBP) and heart rate (HR) were obtained before CCI of the sciatic nerve and 7 days after CCI RCS using a noninvasive tail cuff system (LE 5001 pressure meter, Panlab, Barcelona, Spain). The temperature in the restrainer was maintained at approximately 29–32 °C to ensure that there was an adequate flow of blood through the tail vein. Several blood pressure measurements were obtained from each rat and the mean of three to four values among which the difference was not greater than 10 mm Hg was accepted (Miao et al., 2007).

2.7. Blood samples collection
At the end of the study, rats were anesthetized with thiopental sodium (50 mg/kg) and killed by decapitation. Collected blood samples were centrifuged at 3000 r.p.m. for 10 min and serum stored at −20 °C for further assessment of IL-6 and TNF-α.

2.8. Cytokines serum analysis by ELISA
Cytokines levels in the sera of rats were measured according to the manufacturer’s instructions (Koma Biotech Inc., Seoul, Korea). Briefly, an antibody specific for rat either TNF-α or IL-6 has been coated onto the provided 96 well ELISA microplate. Washing solution was added to each well 3 times. Standards of known rat cytokines concentration and unknown samples were pipetted into these wells. During the first incubation period, i.e. 3 h, the rat TNF-α or IL-6 antigen binds to the coating antibody. After washing, a biotinylated (detection) antibody specific for both cytokines was added. During the second incubation, the detection antibody binds to the rat TNF-α or IL-6 antigens captured during the first incubation. After wash of excess secondary antibody, Streptavidin-horseradish peroxidase (Color Development enzyme) was added. This enzyme binds to the detection antibody. After a third incubation i.e. 30 min and washing to remove all of the unbound enzyme, color development solution was added. Then, the plates were read using a microplate reader at 450 nm. After subtracting the zero reading from each value, a standard curve was created to obtain the concentration of the unknown samples.

2.9. Statistical analysis
The results Data are represented as the group means ± S.E.M. The significance of differences between groups was analyzed using paired Student’s t-test or one-way analysis of variance (ANOVA) followed by the posthoc Dunnett’s test for multiple comparisons as appropriate. All statistical analyses were calculated with Prism software (Graph-Pad Software, version 5, San Diego CA, USA).

3. Results

3.1. Changes in body weight
Body weights were measured at day 0 (pre) and at day 8 (post) for each experimental group. As shown in Table 1, eight days after sciatic nerve ligation alone, chronic exposure to cold or their combination significantly decreased mean body weight when compare pre and post-values. Also, CCI followed by administration of Dex (CCI–Dex group) demonstrated significant decrease between pre- and post-values (P < 0.05, Table 1). While, there were no significant differences in mean body weights of sham RCS, sham CCI and Dex groups prior to procedure at day 0 (pre-values) compared with post-values at day 8.

3.2. Changes in cardiovascular parameters
Preliminary, we investigated the effect of repeated cold stress on systolic blood pressure. When the animals were exposed to RCS at 4 °C daily for 7 days, the systolic blood pressure was significantly higher as compared with the control group of RCS (Sham RCS) starting at day 3 of cold stress (124.0 ± 19 vs. 111.6 ± 1.6, with a continued increase of systolic blood pressure at day 7 (141.0 ± 2.0 vs. 106.7 ± 2.8) of stress. There were no significant differences in systolic blood pressure between control group of RCS (Sham RCS) and sham-operated group (Sham CCI) at different time points (Fig. 1).

Blood pressure and heart rate were measured at day 0 (pre) and at day 8 (post) for each experimental group. As shown in Table 2 eight days after sciatic nerve ligation alone or chronic exposure to cold significantly increased systolic, diastolic and mean blood pressure compared with those of sham controls. Dex significantly decreased only systolic blood pressure compared with sham RCS. In addition, there were significant decrease in both diastolic and mean blood pressure in CCI–Dex compared with CCI group. There were no significant differences between CCI–RCS compared with CCI group in systolic, diastolic or mean blood pressure. The post-changes in heart rate for Dex, CCI, RCS, CCI–RCS group and CCI–Dex groups were significantly changed when compared prevalues (Table 2).

3.3. Changes of nociceptive behaviors
3.3.1. Changes of reaction time in hot plate test
To evaluate acute centrally mediated nociceptive signaling, the hot plate test was employed. Data presented in Table 3 show that treatment of rats with Dex significantly enhanced the reaction time post-value compared with pre-value (P < 0.05). Measurement of reaction time at day 8 (postvalues) compared with day 0 (prevalues), rats in RCS or CCI group showed significantly decreased reaction time on day 8 in the hot plate test.

Administration of Dex to CCI rats failed to increase hot plate latencies when response was evaluated at day 8. However, reaction time at day 8 (postvalues) compared with day 0 (prevalues), rats in the CCI–RCS group showed significantly increased reaction time (P < 0.01).
Table 1
Effect of dexmedetomidine (Dex), chronic constriction injury (CCI), repeated cold stress (RCS) on body weight in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Pre-values</th>
<th>Post-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham RCS</td>
<td>173.8 ± 8.2</td>
<td>179.2 ± 7.5</td>
<td></td>
</tr>
<tr>
<td>Sham CCI</td>
<td>161.0 ± 9.2</td>
<td>164.0 ± 7.9</td>
<td></td>
</tr>
<tr>
<td>Dex</td>
<td>171.5 ± 6.0</td>
<td>173.8 ± 6.8</td>
<td></td>
</tr>
<tr>
<td>CCI</td>
<td>360.5 ± 22.6</td>
<td>332.8 ± 21.9</td>
<td></td>
</tr>
<tr>
<td>RCS</td>
<td>356.3 ± 7.9</td>
<td>317.7 ± 13.2</td>
<td></td>
</tr>
<tr>
<td>CCI + RCS</td>
<td>183.8 ± 11.4</td>
<td>136.5 ± 8.9</td>
<td></td>
</tr>
<tr>
<td>CCI + Dex</td>
<td>313.8 ± 18.6</td>
<td>293.5 ± 20.3</td>
<td></td>
</tr>
<tr>
<td>RCS + Dex</td>
<td>219.2 ± 2.7</td>
<td>198.5 ± 2.4</td>
<td></td>
</tr>
</tbody>
</table>

Day 0 = pre-values and day 8 = post-values.
Chronic constriction injury (CCI), Dexmedetomidine (Dex), repeated cold stress (RCS)
Values are means ± S.E.M. for n = 8 rats per group.
a Significant difference at P < 0.01 and
b Significant difference at P < 0.001 (Student’s t test, compared with pre-values).

Fig. 1. Time-course study of the effect of repeated cold stress on systolic blood pressure in rats. An increase in the systolic blood pressure in cold-stressed rats. Each point represents the mean ± S.E.M. of 8 animals. **P < 0.01 compared with sham RCS. Statistical analysis was done using one way analysis of variance (ANOVA) followed by Dunnett’s test as a posthoc test.

Table 2
Effect of dexmedetomidine (Dex), chronic constriction injury (CCI), repeated cold stress (RCS) on cardiovascular changes heart rate and blood pressure.

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood pressure (mm Hg)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBP</td>
<td>DBP</td>
</tr>
<tr>
<td>Sham RCS</td>
<td>112.9 ± 1.9</td>
<td>79.3 ± 0.8</td>
</tr>
<tr>
<td>Sham CCI</td>
<td>111.0 ± 1.4</td>
<td>72.9 ± 6.3</td>
</tr>
<tr>
<td>Dex</td>
<td>106.4 ± 0.9</td>
<td>72.7 ± 0.8</td>
</tr>
<tr>
<td>CCI</td>
<td>118.3 ± 1.2</td>
<td>94.3 ± 0.6</td>
</tr>
<tr>
<td>RCS</td>
<td>141.4 ± 2.1</td>
<td>116.6 ± 5.5</td>
</tr>
<tr>
<td>CCI + RCS</td>
<td>122.0 ± 2.0</td>
<td>92.5 ± 1.0</td>
</tr>
<tr>
<td>CCI + Dex</td>
<td>120.4 ± 1.0</td>
<td>72.9 ± 0.8</td>
</tr>
<tr>
<td>RCS + Dex</td>
<td>127.9 ± 1.8</td>
<td>94.7 ± 2.9</td>
</tr>
</tbody>
</table>

Day 0 = pre-value and day 8 = post-value.
Systolic (SBP), diastolic (DBP) and mean blood pressure (MBP).
Values are means ± S.E.M. for n = 8 rats per group.

3.3.2. Changes of pain threshold in mechanical hyperalgesia and allodynia induced by CCI

The i.p. administration of Dex (5 μg/kg) did not change significantly the pain threshold compared with pre-value. When the animals were exposed to RCS at 4 °C daily for 7 days, the pain threshold was significantly lower as compared with pre-values in the Randall and Selitto and electronic von Frey tests (Table 3). Similarly, when the left hind paw pain threshold was evaluated on day 8 after ligation of the sciatic nerve and associated exposure to RCS (CCI + RCS), it resulted in the development of mechanical hyperalgesia and allodynia as revealed by a significant decrease in left hind paw pain threshold as compared with preoperative values (Table 3). Whereas, no significant difference in pain threshold was observed on comparing the pre- and post-values of CCI + Dex group (P > 0.05) (Table 3).

3.3.3. Changes of inflammatory mediators

In parallel to effects on cardiovascular parameters and nociceptive behaviors, changes in inflammatory mediators; IL-6 and TNF-α were studied at day 8 in different experimental groups. There was a significant increase in both of IL-6 and TNF-α in CCI compared with control (Sham CCI) group. Compared with CCI group, associated exposure to RCS (CCI + RCS) resulted in a significant decrease IL-6 and TNF-α (Table 4). There was also a significant difference in both IL-6 and TNF-α of the CCI + Dex group compared with CCI group (Table 4).

4. Discussion

Neuropathic pain is well-known as one of the most difficult to treat chronic pain states and presents a significant challenge to clinicians because it often does not respond to conventional analgesic therapies (Rice and Hill, 2006).

We investigated the consequences of repeated cold exposure on neuropathic pain model in rats. Neuropathic pain model was induced in the present study by sciatic nerve ligation in rats. Considering that the sham-operated rats and un-operated hind paw (right paw) pressed normally on the floor and CCI produced a significant hyperalgesia, it was indicated that the CCI of the sciatic nerve used in the current study was successful.

Our data revealed a significant decrease in body weight at day 8 (post-values) in CCI, RCS, CCI + RCS, CCI + Dex and RCS + Dex groups. These results coincide with an in vivo study demonstrating...
In the present study a significant increase in the systolic blood pressure was observed in RCS group. Exposure to cold stress elevates adrenocorticotropic and corticosterone responses, indices of hypothalamo-pituitary–adrenal activity (Bhatnagar and Vining, 2003) as well as the activity of sympathetic nervous system (Ma and Morilak, 2005). Another possible mechanism for increasing the systolic blood pressure is the increase of neuronal activity of a specific brain region, including the paraventricular nucleus of the thalamus, central nucleus of the amygdala and the paraventricular nucleus of the hypothalamus (Kc et al., 2010). In contrast, Simon and Illyes (2001) demonstrated that prolonged overnight cold exposure of rats at 5 °C for 12 weeks causes no blood pressure changes and hence, no activation of sympathetic nervous system. This difference in results may be attributed to the duration of repeated cold stress in their study. Interestingly, in our study the elevated blood pressure was associated with decreased heart rate in RCS group. This relationship between sympathetic and vagal activities was studied clinically during cooling and demonstrated the effect of dexmedetomidine infusion during cold-induced shivering: it was shown that after one h infusion of dexmedetomidine both blood pressure, and systemic catecholamines increased while the heart rate decreased. This suggested preserved parasympathetic modulation of heart rate, while the increased systolic blood pressure and catecholamines due to baroreceptor reflex and increased sympathetic activity (Hogue et al., 2002).

Dexmedetomidine is a potent, highly selective α2 adrenergic agonist, possessing a different specificity for the α2: α1 receptors of 1620:1 (Virtanen et al., 1988). It has sedative, analgesic and anesthetic effects, and sympatholytic properties (Hofer et al., 2005). In the present study, a significant decrease in the diastolic blood pressure, mean blood pressure and heart rate was observed in the CCI+Dex group. Thus, our present findings suggest that the increments in the blood pressure values and heart rate after CCI were due to increased sympathetic nerve activity. As far as our knowledge, no study has been conducted to test the simultaneous effect of cold stress and neuropathic pain on blood pressure and heart rate. Bhatnagar et al. (1998) studied that restraint and intraplantar injection of formalin, following prior exposure to 7 days of intermittent cold in rats increased mean blood pressure and heart rate of either restraint or formalin injected rats. The reason of their finding could be attributed to increased levels of catecholamines (Fukuhara et al., 1996), increased levels of ACTH (Vahl et al., 2005) and change in the dopaminergic neuron activity depending on the nature of the stressor (Valenti et al., 2012).

One of the major findings of the present study is that, a significant decrease in the reaction time and pain threshold when the
animals were exposed to CCI of the sciatic nerve or RCS at 4 °C daily for 7 days, starting at day 4 of cold stress, with a continued decrease of pain threshold at day 8 of cold stress. The inhibition of pain response to stressful stimuli may be mediated by adaptive activation of non-opioid mechanisms including, gamma amino-butyric acid, glutamate, mono-amnergic and endocannabinoid receptors (Ford and Finn, 2008).

Our results showed a significant analgesic effect of Dex observed in the CCI + Dex. Previous work in our laboratory has demonstrated that Dex in the same attempted dose effectively reduced the mechanical allodynia and hyperalgesia after ligation of the left sciatic nerve in rats (Farghaly et al., 2014). The mechanism, by which Dex elicits analgesic and sedative effects, is multi-factorial. Both hypnotic and supraspinial analgesic effects of Dex are mediated by noradrenergic neurons. Dex causes inhibition of norepinephrine release and its neuron associated activity in the descending medullo-spinal noradrenergic pathway and suppresses neuronal activity in the locus coeruleus (Hoy and Keating, 2011).

It has been proposed that irrespective of the characteristic of the pain, whether it is sharp, dull, aching, burning, stabbing, numbness, or tingling, all pains may arise from inflammation. Also, it is known that an increase in the level of proinflammatory cytokines, including TNF-α and IL-6 is an early feature of acute injury (Omougui, 2007). Importantly, our study demonstrated a significant increase in serum levels of IL-6 and TNF-α in CCI group. Based upon several studies, it is postulated that chronic stress and pain resulted in the production of inflammatory molecules (Szebenyi et al., 2000; Can et al., 2008; Bekker et al., 2013). Painful stimulus will lead to sympathetic nervous system activation and hence catecholamine release. Thus, it is reasonable to speculate the return of serum IL-6 and TNF-α to control level associated with Dex in the present experiment. Also, in the current study a significant decrease in serum levels of IL-6 and TNF-α were found in CCI + Dex and CCI + RCS groups compared with CCI group. Dex may also reduce the nuclear translocation and binding activity of activated NF-kB, thus, reducing inflammatory cytokines (Bekker et al., 2013). A recent study demonstrated that Dex, independent of α2-adrenergoreceptor stimulation can reduce the plasma and bronchoalveolar lavage fluid level of IL-6 and TNF-α in cecal ligation and puncture-induced septic rats (Zhang et al., 2015). Surprisingly, in the current study, RCS groups had no difference in both TNF-α and IL-6 plasma levels when compared to corresponding control group (sham RCS). This finding can be explained in terms of adaptation. Only regular winter swimmers had significantly higher concentrations of plasma IL-6, the soluble receptor for IL-6 and cortisol than inexperienced winter swimmers (Dugue and Leppanen, 2000). Moreover, a clinical study demonstrated that IL-6 and TNF-α concentration as measured by ELISA did not change significantly after body cooling due to negative feedback inhibition of IL-6 and TNF-α production (Brazaitis et al., 2014). Also, Luo et al. (2014) showed the existence of these negative relationships between cold stress intensity and the level of IL-6 and TNF-α and IL-8 in bronchoalveolar lavage fluid of Wistar male rats, but, exposure to cold stress at 0°C caused significant increase of these inflammatory cytokines. This could further explain our results of the decrease in serum level of TNF-α and IL-6 in RCS + Dex group. These adaptive responses with long period of repeated stress is not only serve to protect against the source of stress, but also to protect against the animal own normal defense mechanism.

Further investigations are required to better elucidate the clinical application of this study. Applying cold exposure on humans, such as during physiotherapy could be of great benefit in neuropathic pain conditions.

In conclusion

Our study revealed that the analgesic effects of Dex in neuropathic pain model may be, in part, due to inhibition of IL-6 and TNF-α. Importantly, we postulated that cold stress may result in increased resistance to neuropathic pain through decrease production of IL-6 and TNF-α.

Conflict of interest statement

No conflict of interest.

Acknowledgments

We acknowledge the technicians of the Research Pharmacology Laboratory for their help and support.

References


