Neuroscience Letters 510 (2012) 20–23

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

# Intraneural dexamethasone applied simultaneously to rat sciatic nerve constriction delays the development of hyperalgesia and allodynia

Leandro F.S. Bastos[a,b], Daniel C. Medeiros[a], Rafael P. Vieira[c], Linda R. Watkins[b], Márcio M. Coelho[d], Márcio F.D. Moraes[a,*]

[a] Núcleo de Neurociências (NNC), Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Bloco A4, Sala 168, Universidade Federal de Minas Gerais (UFMG), Avenida Antônio Carlos 6627, 31270-901 Belo Horizonte, Brazil[1]
[b] Department of Psychology and Neuroscience, Muenzinger Building, Colorado Avenue, University of Colorado at Boulder, Boulder, 80309 CO, USA
[c] Departamento de Química, Instituto de Ciências Exatas, UFMG, Brazil
[d] Laboratório de Farmacologia, Departamento de Produtos Farmacêuticos, Faculdade de Farmácia, UFMG, Brazil

ARTICLE INFO

*Article history:*
Received 13 October 2011
Received in revised form 8 December 2011
Accepted 28 December 2011

*Keywords:*
Chronic constriction injury
Neuropathic pain
Peripheral neuropathy
Inflammation
Neuroimmune interaction
Corticosteroid

ABSTRACT

Although neuroimmune interactions associated with the development of pain sensitization in models of neuropathic pain have been widely studied, there are some aspects that require further investigation. Thus, we aimed to evaluate whether the local intraneural or perineural injections of dexamethasone, an efficacious anti-inflammatory and immunosuppressant drug, delays the development of both thermal hyperalgesia and mechanical allodynia in an experimental model of neuropathic pain in rats. Hargreaves and electronic von Frey tests were applied. The chronic constriction injury (CCI) of right sciatic nerve was performed. Single intraneural dexamethasone administration at the moment of constriction delayed the development of sensitization for thermal hyperalgesia and mechanical allodynia. However, perineural administration of dexamethasone, at the highest dose, did not delay experimental pain development. These results show that inflammation/immune response at the site of nerve lesion is an essential trigger for the pathological changes that lead to both hyperalgesia and allodynia. In conclusion, this approach opens new opportunities to study cellular and molecular neuroimmune interactions associated with the development of pain derived from peripheral neuropathies.

© 2012 Elsevier Ireland Ltd. All rights reserved.

## 1. Introduction

Immune activation and the subsequent release of immune mediators in the peripheral nervous system contribute to pain. A variety of peripheral immune cells, including mast cells, macrophages, and lymphocytes, are resident in peripheral nerves and/or recruited to sites of peripheral nerve injury [23,24]. These cells produce a plethora of inflammatory mediators that sensitize or directly activate peripheral nerves to generate action potentials, leading to peripheral and central sensitization [4].

Additional support to a role played by neuroinflammation in the development of experimental pain arises from the finding that dexamethasone, a potent anti-inflammatory and immunosuppressant drug, inhibits fibrovascular tissue formation around sutures, infiltration of inflammatory cells and thermal hyperalgesia when administered intraperitoneally before and after CCI in rats [3]. It is tempting to associate the antihyperalgesic effect induced by dexamethasone to its action at the site of the nerve injury, leading to reduced infiltration of inflammatory cells and production of inflammatory mediators. However, when glucocorticoids are administered systemically, the site(s) of their action is(are) not clear as it has been shown that these drugs may also induce experimental pain suppressive effects after intrathecal administration in experimental models of neuropathic pain [12,25]. However, when rats are subjected to CCI and have the sciatic nerves re-exposed and treated locally with a depot form of methylprednisolone at day 10 after injury, antiallodynic and antihyperalgesic effects are observed up to day 21 [9], thus providing evidence that glucocorticoids may act locally at the site of nerve injury to inhibit sensitization. These results indicate that glucocorticoids applied around the site of nerve injury may reverse the sensitization when this phenomenon is fully established. To further elucidate the site of action of glucocorticoids, in the present study we investigated the ability of intraneural *versus* perineural administration of dexamethasone to prevent the development of thermal hyperalgesia and mechanical allodynia, when the drug was delivered at the time of sciatic injury in rats.

*Abbreviations:* CCI, chronic constriction injury; CGRP, calcitonin gene-related peptide; DRG, dorsal root ganglion.

* Corresponding author. Tel.: +55 31 3409 2930; fax: +55 31 3409 2924.
*E-mail address:* mfdm@icb.ufmg.br (M.F.D. Moraes).

[1] Permanent address.

0304-3940/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2011.12.062

## 2. Materials and methods

### 2.1. Animals

All experiments were performed according to the ethical guidelines for the investigation of experimental pain in conscious animals [28] and approved by the Ethics Committee on Animal Experimentation of the Federal University of Minas Gerais (protocol # 168/10). Male Wistar rats (240–280 g) were used, with groups matched on body mass (approximately 260 g). Efforts were made to minimize both animal distress and the number of animals used. The animals had free access to food and water and were maintained in a room with a 12 h light–dark cycle for 3 days before the experiment to allow for acclimatization. The experiments were conducted in the middle of light circadian phase, at room temperature between 26 and 28 °C, which corresponds to the thermoneutral zone for rats [7]. For three consecutive days before the experiment, the animals were habituated for approximately 30 min to the experimental apparatuses in order to minimize stress-induced antinociception. The investigator performing behavioral tests was blind to treatments.

### 2.2. Surgery and drug administration

CCI of the right sciatic nerve was performed as previously described by Bennett and Xie [2], under anesthesia induced by ketamine (90 mg/kg) and xylazine (9 mg/kg) hydrochlorides (both manufactured by Vetbrands; Paulínia, São Paulo, Brazil). Four ligatures with a flexible nylon thread (Sanifill, Rio de Janeiro, Brazil) were tied loosely around the sciatic nerve at mid-thigh level [13]. Sham-operated animals underwent the same surgical procedure, save that the sciatic was not ligated. When dexamethasone (100 or 250 μg/kg assuming 260 g rat on average hence 26 or 65 μg/rat; Sigma–Aldrich, St. Louis, MO, USA) or equivolume (10 μl) saline vehicle was injected directly into the sciatic nerve or administered perineurally onto the nerve, the two outermost ligatures were placed first and injection was performed using a 30-gage needle connected to a Hamilton syringe (Reno, NV, USA) in the center between the ligatures. Then, the remaining middle two sutures were placed, as previously described [21].

### 2.3. Evaluation of thermal hyperalgesia

Paw withdrawal latency to a thermal stimulus was measured with a plantar test unit (model 7370, Ugo Basile, Comerio, Italy). Briefly, rats were kept in Perspex cells having a glass pane as a floor and a source of infrared light was directed upwards from below the glass toward the right hind paw plantar surface [8]. Cut-off time (20 s) was imposed to avoid tissue damage. The value for the response was obtained by averaging three measurements, with each determination separated by a minimum of 20 s. These procedures were performed under the same laboratory conditions as previously described [16].

### 2.4. Evaluation of mechanical allodynia

Rats were placed in acrylic cages with wire mesh floors 15 min before the start of testing. The test consisted of evoking a hind paw flexion reflex with a hand-held force transducer (electronic pressure-meter, Insight, Ribeirão Preto, Brazil) adapted to a 0.5-mm$^2$ polypropylene tip. The experimenter was trained to apply the tip perpendicularly to the central area of the hind paw with a gradual increase in pressure. The endpoint was characterized by the removal of the paw usually followed by flinching movements. After the paw withdrawal, the intensity of the pressure was automatically recorded. The value for the response was obtained by averaging five measurements. The animals were tested before and after surgeries and/or treatments. The results are expressed by the absolute withdrawal threshold (in grams). These procedures were similar to those previously described [20].

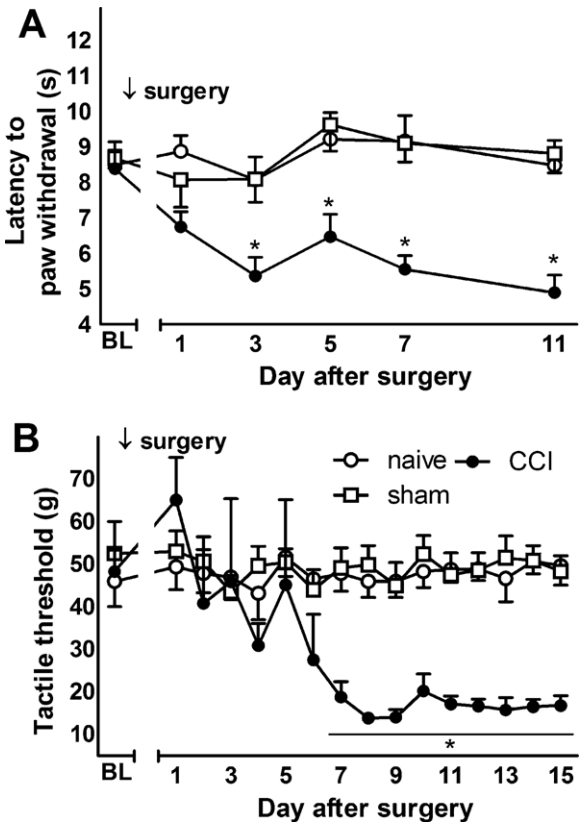



**Fig. 1.** Effect induced by chronic constriction injury (CCI) on the development of thermal hyperalgesia (A) and mechanical allodynia (B) in rats. Data are expressed as mean ± standard error of the mean ($n$ = 5–7). *$p$ < 0.05 compared with the vehicle-treated group at the respective time-point, tested by two-way repeated measures ANOVA followed by Bonferroni *post hoc* test, taking time and surgery as main factors. Naive and sham-operated animals were unaffected by the surgery factor at all time points.

### 2.5. Statistical analyses

The results, expressed as mean ± standard error of the mean, were analyzed by two-way repeated measures ANOVA followed by Bonferroni *post hoc* test, taking surgery and time (Fig. 1A and B) or treatment and time (Figs. 2A, B and 3A, B) as main effects. Values of $p < 0.05$ were considered to show significant differences between means. The software GraphPad Prism® 5.0 (San Diego, CA, USA) was used for the analyses.

## 3. Results

Thermal hyperalgesia was observed from day 3 after surgery onward (Fig. 1A; main effect of surgery factor: $F_{(2,100)} = 21.68$, $p < 0.0001$; main effect of time: $F_{(5,100)} = 2.64$, $p = 0.0275$; interaction: $F_{(10,100)} = 1.87$, $p = 0.0588$), whereas mechanical allodynia was observed from day 7 onward (Fig. 1B; main effect of surgery factor: $F_{(2,240)} = 10.66$, $p = 0.0011$; main effect of time: $F_{(15,240)} = 3.95$, $p < 0.0001$; interaction: $F_{(30,240)} = 3.01$, $p < 0.0001$).

Next, it was tested whether a single intraneural injection of dexamethasone at the time of sciatic injury could delay the development of both thermal hyperalgesia and mechanical allodynia. We

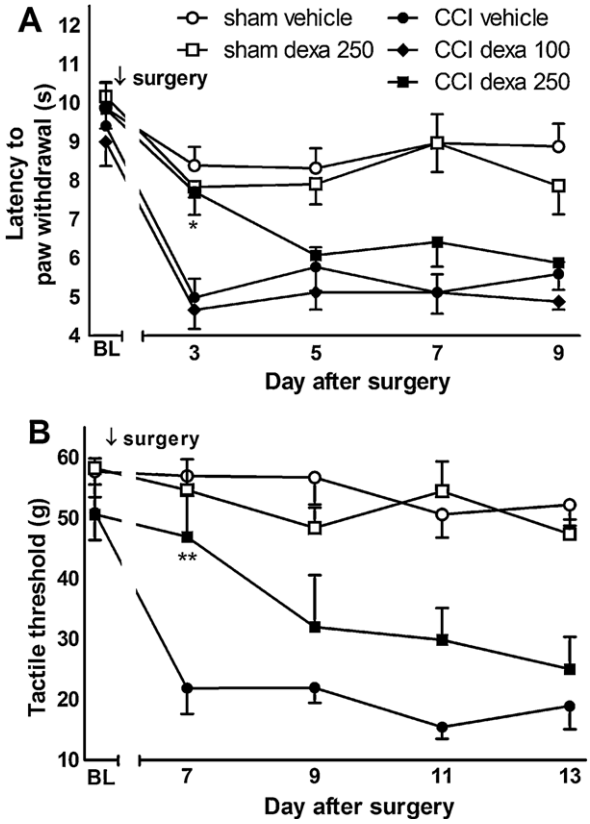



**Fig. 2.** Intraneural dexamethasone (dexa; 100 or 250 μg/kg, 10 μl) at the moment of chronic constriction injury (CCI) delayed the development of thermal hyperalgesia (A) and mechanical allodynia (B) in rats. Data are expressed as mean ± standard error of the mean ($n$ = 5–8). *$p$ < 0.05 and **$p$ < 0.01 compared with the vehicle-treated group at the respective time-point, tested by repeated measures two-way ANOVA followed by Bonferroni *post hoc* test, taking time and treatment as main factors. Sham-operated animals were unaffected by treatment at all time points.

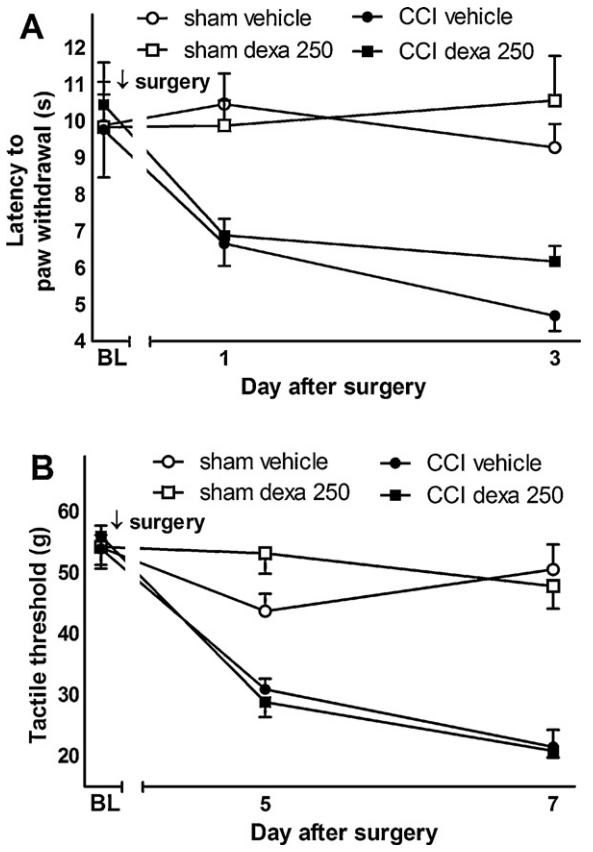



**Fig. 3.** Perineural dexamethasone (dexa; 250 μg/kg, 10 μl) at the moment of chronic constriction injury (CCI) did not delay the development of thermal hyperalgesia (A) and mechanical allodynia (B) in rats ($p$ > 0.05). Data are expressed as mean ± standard error of the mean ($n$ = 7–8) and tested by repeated measures two-way ANOVA followed by Bonferroni *post hoc* test, taking time and treatment as main factors. Sham animals were also unaffected by treatment at all time-points ($p$ > 0.05).

observed that intraneural treatment with the highest (250 μg/kg) dose of dexamethasone led to a rightward shift in the curve of sensitization for thermal (Fig. 2A; main effect of treatment: $F_{(2,68)} = 5.13$, $p = 0.0181$; main effect of time: $F_{(4,68)} = 21.08$, $p < 0.0001$; interaction: $F_{(8,68)} = 0.88$, $p = 0.5346$) and mechanical (Fig. 2B; main effect of treatment: $F_{(1,52)} = 4.82$, $p = 0.0468$; main effect of time: $F_{(4,52)} = 18.94$, $p < 0.0001$; interaction: $F_{(4,52)} = 3.01$, $p = 0.0261$) stimuli. Sham-operated animals had their thermal (Fig. 2A; main effect of treatment: $F_{(1,56)} = 0.47$, $p = 0.5060$; main effect of time: $F_{(4,56)} = 4.97$, $p = 0.0017$; interaction: $F_{(4,56)} = 0.50$, $p = 0.7371$) and mechanical (Fig. 2B; main effect of treatment: $F_{(1,56)} = 0.48$, $p = 0.4977$; main effect of time: $F_{(4,56)} = 1.75$, $p = 0.1528$; interaction: $F_{(4,56)} = 0.95$, $p = 0.4398$) sensitivities unaffected by treatment over the time course evaluated (Fig. 2A and B).

Finally, when the administration of dexamethasone (250 μg/kg) was performed perineurally, neither antihyperalgesic (main effect of treatment: $F_{(1,22)} = 1.28$, $p = 0.2814$; main effect of time: $F_{(2,22)} = 17.91$, $p < 0.0001$; interaction: $F_{(2,22)} = 0.31$, $p = 0.7342$) nor antiallodynic (main effect of treatment: $F_{(1,22)} = 0.01$, $p = 0.9415$; main effect of time: $F_{(2,22)} = 78.72$, $p < 0.0001$; interaction: $F_{(2,22)} = 0.27$, $p = 0.7628$) effects were observed in CCI rats (Fig. 3). Likewise, both thermal (main effect of treatment: $F_{(1,24)} = 0.10$, $p = 0.7626$; main effect of time: $F_{(2,24)} = 0.06$, $p = 0.9446$; interaction: $F_{(2,24)} = 0.49$, $p = 0.6213$) and mechanical (main effect of treatment: $F_{(1,24)} = 0.52$, $p = 0.4866$; main effect of time: $F_{(2,24)} = 2.16$, $p = 0.1374$; interaction: $F_{(2,24)} = 2.28$, $p = 0.1237$) sensitivities of sham-operated rats were unaffected by treatment over the time course evaluated (Fig. 3).

## 4. Discussion and conclusions

In the present study, we show that the intraneural, but not perineural, administration of dexamethasone (250 μg/kg based on 260 g rats, on average) delays the development of both thermal hyperalgesia and mechanical allodynia in CCI rats when administered at the time of sciatic nerve injury. While not examined here, we predict that intraneural dexamethasone, but not perineural dexamethasone, inhibited the infiltration of inflammatory cells into the nerve and may have also inhibited the production of inflammatory mediators by Schwann cells. Suppression of neuropathic pain development by intraneural dexamethasone cannot be accounted for by systemic spread, given that perineural dexamethasone would more readily become systemic, yet perineural dexamethasone failed to affect the development of neuropathic pain. Furthermore, the fact that different time courses were observed for intraneural dexamethasone effects on hyperalgesia and allodynia reinforces prior evidence that CCI-induced thermal hyperalgesia and mechanical allodynia have distinct underlying mechanisms [4].

It has previously been shown that dexamethasone has a 2.3 h half-life after either intravenous or intramuscular injection in rats [18]. However, no information is available about the pharmacokinetics of this drug after intraneural administration. If one assumes that dexamethasone exhibits similar pharmacokinetics after systemic *versus* intraneural administration, it would then be highly likely that dexamethasone had already been biotransformed and eliminated by the time that antiallodynic effects were observed.

If this is true, then these results support that a short-lasting anti-inflammatory, immunosuppressant treatment, administered at the time of nerve injury, can be sufficient to delay experimental pain development for days. Consistent with our findings, local administration of the glucocorticoid agonist triamcinolone to ferret lingual nerve at the time of injury reduces injury-enhanced, evoked electrical activity in the nerve as long as 3 months after the injury [27].

It is likely that dexamethasone exerts its antihyperalgesic and antiallodynic effects through multiple mechanisms. The proinflammatory responses of neutrophils, macrophages, lymphocytes and mast cells, which normally infiltrate into the nerve after injury [14,15], can be suppressed by dexamethasone [3,5,10,11,26]. Moreover, Schwann cells are activated after CCI [6], and their production of cytokines and chemokines is inhibited by dexamethasone *in vitro* [17], though *in vivo* evidence seems to be lacking. These various cell types can release a wide array of inflammatory mediators which sensitize or activate nociceptive neurons, thereby enhancing pain [4]. Given the pleiotropic genomic and non-genomic anti-inflammatory actions of dexamethasone [1], it may prevent the initial proinflammatory events initiated in response to CCI and thus prevent or delay the subsequent development of hyperalgesia or allodynia.

Besides actions on immune cells, both genomic and non-genomic actions on neurons may be hypothesized to explain the effect induced by intraneural dexamethasone on the development of hyperalgesia and allodynia. Supporting genomic actions, modulation of expression of channels involved in excitability of neurons in nociceptive pathways by glucocorticoids may also interfere with nociceptive responses [22]. Dexamethasone may alter expression of pain-relevant receptors, as prenatal exposure of rats to dexamethasone decreases P2X3 expression in DRG and spinal cord [22], though effect on P2X3 expression in adult rats remains to be tested. Furthermore, glucocorticoids can alter the expression of neurotransmitters implicated in pain transmission. For example, removal of endogenous glucocorticoids by adrenalectomy increases calcitonin gene-related peptide (CGRP) concentrations in the dorsal root ganglia (DRG) suggestive that increases in glucocorticoids may be able to suppress CGRP production. As the adrenalectomy-induced increases in CGRP were reversed by dexamethasone [19], this give credence to the idea that glucocorticoids, rather than other adrenal factors, underlie the effects observed.

In conclusion, the results reinforce the evidence that early neuroimmune interactions are essential for triggering mechanisms necessary for the development of long lasting thermal hyperalgesia and mechanical allodynia in models of neuropathic pain, though additional actions on neural excitability may also be taken into account. The study presents a localized methodological approach that opens opportunities to study in detail the cellular and molecular multi-step inflammatory processes by which peripheral neuropathies lead to persistent, pathological pain states.

## Conflict of interest

The authors state no actual or potential conflict of interest.

## Acknowledgments

We acknowledge Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Ministry of Education, Brazil), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Ministry of Science and Technology, Brazil) and INCT-INCeMaq, Fundação de Apoio à Pesquisa do Estado de Minas Gerais (FAPEMIG, Minas Gerais, Brazil) and Pró-Reitoria de Pesquisa (PRPq) da UFMG for constant support.

## References

[1] P.J. Barnes, Corticosteroid effects on cell signalling, Eur. Respir. J. 27 (2006) 413–426.
[2] G.J. Bennett, Y.K. Xie, A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man, Pain 33 (1988) 87–107.
[3] A.L. Clatworthy, P.A. Illich, G.A. Castro, E.T. Walters, Role of peri-axonal inflammation in the development of thermal hyperalgesia and guarding behavior in a rat model of neuropathic pain, Neurosci. Lett. 184 (1995) 5–8.
[4] M. Costigan, J. Scholz, C.J. Woolf, Neuropathic pain a maladaptive response of the nervous system to damage, Annu. Rev. Neurosci. (2009).
[5] D. Franchimont, J. Galon, M. Gadina, R. Visconti, Y. Zhou, M. Aringer, D.M. Frucht, G.P. Chrousos, J.J. O'Shea, Inhibition of Th1 immune response by glucocorticoids: dexamethasone selectively inhibits IL-12-induced Stat4 phosphorylation in T lymphocytes, J. Immunol. 164 (2000) 1768–1774.
[6] E.S. Fu, Y.P. Zhang, J. Sagen, K.A. Candiotti, P.D. Morton, D.J. Liebl, J.R. Bethea, R. Brambilla, Transgenic inhibition of glial NF-kappa B reduces pain behavior and inflammation after peripheral nerve injury, Pain 148 (2010) 509–518.
[7] C.J. Gordon, Thermal biology of the laboratory rat, Physiol. Behav. 47 (1990) 963–991.
[8] K. Hargreaves, R. Dubner, F. Brown, C. Flores, J. Joris, A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia, Pain 32 (1988) 77–88.
[9] A. Johansson, G.J. Bennett, Effect of local methylprednisolone on pain in a nerve injury model. A pilot study, Regional Anesth. 22 (1997) 59–65.
[10] M. Kamberi, E. Brummer, D.A. Stevens, Regulation of bronchoalveolar macrophage proinflammatory cytokine production by dexamethasone and granulocyte–macrophage colony-stimulating factor after stimulation by *Aspergillus conidia* or lipopolysaccharide, Cytokine 19 (2002) 14–20.
[11] O. Kassel, A. Sancono, J. Kratzschmar, B. Kreft, M. Stassen, A.C. Cato, Glucocorticoids inhibit MAP kinase via increased expression and decreased degradation of MKP-1, EMBO J. 20 (2001) 7108–7116.
[12] Z.L. Ma, W. Zhang, X.P. Gu, W.S. Yang, Y.M. Zeng, Effects of intrathecal injection of prednisolone acetate on expression of NR2B subunit and nNOS in spinal cord of rats after chronic compression of dorsal root ganglia, Ann. Clin. Lab. Sci. 37 (2007) 349–355.
[13] P.G. Motta, A.C. Perez, D.P. Alves, I.D. Duarte, Peripheral control of inflammatory but not neuropathic pain by endogenous cholinergic system, Pharmacology 88 (2011) 18–25.
[14] T. Okuda, O. Ishida, Y. Fujimoto, N. Tanaka, A. Inoue, Y. Nakata, M. Ochi, The autotomy relief effect of a silicone tube covering the proximal nerve stump, J. Orthop. Res. 24 (2006) 1427–1437.
[15] N.M. Perkins, D.J. Tracey, Hyperalgesia due to nerve injury: role of neutrophils, Neuroscience 101 (2000) 745–757.
[16] L.T. Rocha, K.A. Costa, A.C. Oliveira, E.B. Nascimento, C.M. Bertollo Jr., F. Araujo, L.R. Teixeira, S.P. Andrade, H. Beraldo, M.M. Coelho, Antinociceptive, antiedematogenic and antiangiogenic effects of benzaldehyde semicarbazone, Life Sci. 79 (2006) 499–505.
[17] J.L. Rutkowski, G.F. Tuite, P.M. Lincoln, P.J. Boyer, G.I. Tennekoon, S.L. Kunkel, Signals for proinflammatory cytokine secretion by human Schwann cells, J. Neuroimmunol. 101 (1999) 47–60.
[18] M.N. Samtani, W.J. Jusko, Comparison of dexamethasone pharmacokinetics in female rats after intravenous and intramuscular administration, Biopharm. Drug Dispos. 26 (2005) 85–91.
[19] G.D. Smith, J.R. Seckl, W.J. Sheward, J.G. Bennie, S.M. Carroll, H. Dick, A.J. Harmar, Effect of adrenalectomy and dexamethasone on neuropeptide content of dorsal root ganglia in the rat, Brain Res. 564 (1991) 27–30.
[20] G.G. Vivancos, W.A. Verri, T.M. Cunha Jr., I.R. Schivo, C.A. Parada, F.Q. Cunha, S.H. Ferreira, An electronic pressure-meter nociception paw test for rats, Braz. J. Med. Biol. Res. 37 (2004) 391–399.
[21] R. Wagner, M. Janjigian, R.R. Myers, Anti-inflammatory interleukin-10 therapy in CCI neuropathy decreases thermal hyperalgesia, macrophage recruitment, and endoneurial TNF-alpha expression, Pain 74 (1998) 35–42.
[22] H. Wang, M. Zhou, B. Cong, P. He, M. Xu, X. Ni, B. Ma, Transient changes in P2X3 but not TRPV1 mRNA expression in rat after prenatal exposure to glucocorticoids, Auton. Neurosci. 141 (2008) 112–116.
[23] L.R. Watkins, M.R. Hutchinson, E.D. Milligan, S.F. Maier, "Listening" and "talking" to neurons: implications of immune activation for pain control and increasing the efficacy of opioids, Brain Res. Rev. 56 (2007) 148–169.
[24] L.R. Watkins, S.F. Maier, Beyond neurons: evidence that immune and glial cells contribute to pathological pain states, Physiol. Rev. 82 (2002) 981–1011.
[25] W. Xie, X. Liu, H. Xuan, S. Luo, X. Zhao, Z. Zhou, J. Xu, Effect of betamethasone on neuropathic pain and cerebral expression of NF-kappaB and cytokines, Neurosci. Lett. 393 (2006) 255–259.
[26] H. Yarwood, S. Nourshargh, S. Brain, T.J. Williams, Effect of dexamethasone on neutrophil accumulation and oedema formation in rabbit skin: an investigation of site of action, Br. J. Pharmacol. 108 (1993) 959–966.
[27] J.M. Yates, K.G. Smith, P.P. Robinson, The effect of triamcinolone hexacetonide on the spontaneous and mechanically-induced ectopic discharge following lingual nerve injury in the ferret, Pain 111 (2004) 261–269.
[28] M. Zimmermann, Ethical guidelines for investigations of experimental pain in conscious animals, Pain 16 (1983) 109–110.