Int.J.Curr.Microbiol.App.Sci (2020) *9*(10): 4077-4097

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 9 Number 10 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.910.468

Nitric Oxide and its Modulators in Chronic Constriction Injury-Induced Neuropathic Pain in Sprague-Dawley Rats

A. K. Naik*

College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology (O.U.A.T.), Bhubaneswar, India

**Corresponding author*

Keywords

Neuropathic pain, Nitric oxide, Chronic constriction injury, NOS inhibitors

Article Info

Accepted:
04 September 2020
Available Online:
10 October 2020

A B S T R A C T

This study investigated the involvement of nitric oxide (NO) in peripheral neuropathy produced by chronic constriction injury (CCI) of the sciatic nerve in Sprague-Dawley rats, using NO precursors, NO donors, and nitric oxide synthase (NOS) inhibitors. CCI produced marked neuropathic pain, as demonstrated by enhanced mechanical, thermal, and cold allodynia in nociceptive behavioural assays. Administration of the NO precursor L-arginine and the NO donor's sodium nitroprusside and S-nitroso-N-acetyl penicillamine significantly intensified hyperalgesia and allodynia, indicating a pro-nociceptive role of NO in neuropathic conditions. Intracerebroventricular administration of NOS inhibitors, including L-NG-nitroarginine methyl ester, N-iminoethyl-L-lysine, and 7-nitroindazole, did not alter pain responses, whereas their intraperitoneal administration—particularly aminoguanidine, L-NG-nitroarginine methyl ester, and 7-nitroindazole—significantly reduced neuropathic pain. Elevated nitrate and nitrite levels in the ligated sciatic nerve further suggest local up-regulation of NO contributing to the development and persistence of neuropathic pain. Overall, these findings demonstrate that both endogenous and exogenous NO play a critical role in CCI-induced neuropathy, highlighting modulation of the L-arginine–NO pathway as a potential therapeutic strategy for neuropathic pain management.

Introduction

Neuropathic pain arises from a primary lesion or functional disturbance of the nervous system, which may involve peripheral components (peripheral nerves, plexus, or nerve roots) or central structures. The painful region corresponding to the damaged nerve territory typically exhibits allodynia (heightened sensitivity to normally innocuous stimuli), hyperalgesia, and hyperpathia, which are recognized as characteristic features of neuropathic pain (Gordh, 1998). Lesions responsible for neuropathic pain may occur at various anatomical levels, most commonly affecting peripheral nerves, nerve plexuses, dorsal nerve roots, the spinal cord, or the brain. Despite extensive research, the mechanisms underlying neuropathic pain remain incompletely understood. Several experimental models of peripheral mononeuropathy have been developed in

Sprague-Dawley rats, including chronic constriction injury (Bennett and Xie, 1988), partial sciatic nerve ligation (Seltzer et al., 1990), and injury to the sciatic nerve root (Kim and Chung, 1992). The development of allodynia and hyperalgesia following these injuries has significantly contributed to advances in understanding nerve injury–induced neuropathic pain. Alterations in pain behaviour have been reported to peak between 2 and 4 weeks after nerve injury (Basile et al., 1993).

Multiple pathological processes are believed to be involved in nerve injury–associated pain. In view of this, the present study was undertaken to evaluate the role of nitric oxide (NO) using the NO precursor L-arginine; NO donors such as sodium nitroprusside and S-nitroso-N-acetyl penicillamine; and nitric oxide synthase (NOS) inhibitors including L-NG-nitroarginine methyl ester, N-iminoethyl-L-lysine, 7-nitroindazole, and aminoguanidine, in modulating peripheral neuropathy and pain-related behavioural responses in Sprague-Dawley rats subjected to chronic constriction injury.

Neuropathic pain is considerably more difficult to manage than nociceptive pain. In individual patients, it is often chronic, spontaneous, and/or stimulus-evoked, and in many cases remains refractory to treatment (Gordh, 1998). Despite substantial progress in understanding the neurobiology of chronic pain, effective pharmacological management remains limited, and the underlying mechanisms are still poorly defined (Tsuda et al., 2003). Neuropathic pain is unlikely to result from a single pathophysiological mechanism (Malmberg and Basbaum, 1998). It frequently shows poor responsiveness to conventional analgesics such as opioids and non-steroidal anti-inflammatory drugs (Tanelian and Brose, 1991; Rowbotham, 1994). The use of opioids in neuropathic pain remains controversial due to their reduced efficacy compared to other pain conditions.

Pharmacological approaches currently under investigation for neuropathic pain management include N-methyl-D-aspartate (NMDA) receptor antagonists, adenosine analogues, neuron-specific Ca^{2+} channel blockers, and NO-modulating agents (Gordh, 1998). Activation of NMDA receptors leads to increased intracellular Ca^{2+} levels and stimulation of Ca^{2+}-dependent protein kinase C, which in turn promotes NO synthesis, contributing to sustained pain sensitization. Emerging evidence suggests that hyperalgesia in chronic pain may involve not only NO itself but also peroxynitrite, a reactive product formed from the interaction of NO with superoxide radicals (Tal, 1996). Accordingly, the present study aims to elucidate the role of NO in neuropathy induced by chronic constriction injury of the sciatic nerve in Sprague-Dawley rats.

Materials and Methods

Animals

Adult male albino Sprague-Dawley rats of the Wistar strain, weighing 175–225 g, procured from the Laboratory Animal Resource Section of the Indian Veterinary Research Institute, were used in this investigation. The animals were housed in groups of 5–6 per colony cage for one week prior to the induction of chronic constriction injury (CCI) of the sciatic nerve. Following surgery, Sprague-Dawley rats were maintained individually in cages at a controlled room temperature of 25 ± 2 °C. During this period, the animals were gently and repeatedly handled to facilitate acclimatization to laboratory conditions and minimize stress. A standard balanced rat diet supplied by the Feed Technology Unit of the Institute and potable drinking water were made available ad libitum. All experimental

protocols were reviewed and approved by the Institutional Animal Ethics Committee.

Induction of chronic constriction injury

Sprague-Dawley rats selected for surgery were fasted for 12 h prior to the procedure. Anesthesia was induced using ketamine hydrochloride (100 mg/kg, i.m.), after which the hair around the mid-thigh region was clipped and shaved. Chronic constriction injury of the sciatic nerve was produced according to the method described by Bennett and Xie (1988). The common sciatic nerve of the right hind limb was exposed at the mid-thigh level by blunt dissection through the biceps femoris muscle. Approximately 7 mm of the nerve, proximal to the trifurcation, was carefully freed from surrounding connective tissue. Four loose ligatures (4-0 silk) were placed around the nerve at intervals of about 1 mm, resulting in a constricted segment measuring 4–5 mm in length. The degree of constriction was adjusted to reduce, but not completely block, epineural blood flow. The surgical incision was closed in layers. In sham-operated animals, the same surgical exposure was performed without ligation of the sciatic nerve. After skin closure, povidone-iodine solution was applied topically, and oxytetracycline (Terramycin®, Pfizer, India) was administered intramuscularly at a dose of 50 mg/kg body weight for three consecutive days as a prophylactic measure against infection. Operated Sprague-Dawley rats were housed individually with free access to food and water and allowed a recovery period of two weeks prior to drug administration or nerve excision.

Intracerebroventricular (I.C.V.) cannulation

Intracerebroventricular cannulation was carried out in experimental Sprague-Dawley rats as described by Verster et al. (1971), under strict aseptic conditions. Animals were deprived of food for 12 h before surgery and anesthetized with ketamine hydrochloride (100 mg/kg, a.m.). A midline incision of approximately 3 cm was made along the skull, extending from anterior to posterior regions. A small burr hole was drilled at coordinates 2 mm lateral and 1 mm posterior to the bregma on the right side. Two stainless-steel screws were anchored to the skull, positioned 1 mm anterior and 1 mm posterior to the bregma, ensuring they did not penetrate the cranial cavity. A polyethylene guide cannula (No. 47), prefilled with artificial cerebrospinal fluid (a CSF), was inserted through the burr hole to a depth of 4 mm below the skull surface. The cannula was secured using dental cement and the stainless-steel screws. The internal volume of the cannula was 5 µl. To confirm patency, 5 µl of a CSF was infused into the right lateral ventricle, after which the cannula opening was sealed by gentle heat. The animals were allowed to recover for 7 days and were subsequently divided into groups of six for control and pharmacological treatments. At the end of the experimental period, correct placement of the cannula was verified by injecting 5 µl of 1% Evans blue dye intracerebroventricularly and examining dye distribution within the right lateral ventricle.

I.C.V. administration of drugs

Drug solutions were freshly prepared immediately before intracerebroventricular administration. All compounds were dissolved in sterile aCSF, except 7-nitroindazole, which was prepared in arachis oil. Drug concentrations were adjusted so that a uniform volume of 5 µl was administered intracerebroventricularly at a rate of 1 µl/min using a 10 µl syringe, after cutting open the sealed cannula tip. This was followed by an additional 5 µl of aCSF to ensure complete

delivery of the drug into the ventricle, after which the cannula was resealed. Control animals received 5 µl of aCSF or arachis oil alone. The doses of NO modulators employed in the study are listed in Table 1.

Table 1. Intracerebroventricular (i.c.v.) and intraperitoneal (i.p.) doses of nitric oxide (NO) modulators

Category	NO modulator	i.c.v. dose (µg/rat)	i.p. dose (mg/kg)
NO inhibitors	*L*-N^G^-nitroarginine methyl ester	50, 100, 200	3, 10, 30
	N-iminoethyl lysine	50, 100, 200	–
	7-nitroindazole	50, 100, 200	3, 10, 30
	Aminoguanidine	–	30, 100, 300
NO precursor	*L*-arginine	–	0.3, 0.5, 1.0
NO donors	Sodium nitroprusside	–	0.3, 1.0, 3.0
	S-nitroso-*N*-acetyl penicillamine	–	0.3, 1.0, 3.0

Recording of pain threshold

Mechanical stimulation

Mechanical pain threshold was assessed using the Randall–Selitto method (1957) with a Randall–Selitto analgesia meter (UGO Basile, Varese, Italy). Measurements were recorded immediately before drug administration (0 h) and at 1, 3, 5, and 7 h thereafter in Sprague-Dawley rats subjected to chronic constriction injury. A cut-off force of 150 g was maintained. Changes in pain threshold in treated groups were compared with corresponding cerebrospinal fluid-treated CCI control groups. Results are expressed as mean pressure (g) ± S.E.M.

Radiant heat

Thermal nociceptive threshold was determined using a radiant heat apparatus (UGO Basile, Varese, Italy). Paw withdrawal latency was measured in seconds immediately prior to (0 h) and at 1, 3, 5, and 7 h following drug administration. The paw was placed on the heat source, and the time taken for withdrawal was recorded. A cut-off latency of 15 s was applied. Data are presented as mean withdrawal latency (s) ± S.E.M. and compared with corresponding control values.

Cold allodynia

Cold allodynia was evaluated by immersing the right hind paw of CCI-induced

Sprague-Dawley rats in ice-cold water maintained at 4 ± 1 °C. Paw withdrawal latency was measured immediately before (0 h) and at 1, 3, 5, and 7 h after drug administration. Treated groups were compared with aCSF-treated CCI control Sprague-Dawley rats at corresponding time points. A cut-off time of 20 s was used, and results are expressed as mean latency (s) ± S.E.M.

Estimation of nitrate/nitrite

Sciatic nerve samples were collected from CCI-induced and sham-operated Sprague-Dawley rats on the 15th postoperative day. A segment approximately 1.5 cm in length, extending 5 mm proximal and distal to the injury site, was excised and homogenized at a dilution of 1:100 for nitrate and nitrite estimation. Blood samples were also collected for serum separation. Nitrate and nitrite levels in nerve homogenates and serum were quantified according to the method described by Sastry et al. (2002).

The assay involves reduction of nitrate to nitrite using a copper–cadmium alloy, followed by color development with Griess reagent (sulfanilamide and N-1-naphthyl ethylenediamine) under acidic conditions. Concentrations were calculated using a standard curve and expressed as µmol/g of tissue or µmol/ml of serum.

Statistical analysis

Data obtained from NO modulator treatments and nitrate/nitrite estimations were analyzed using analysis of variance (ANOVA), followed by the Studentized range test. A value of $p < 0.05$ was considered statistically significant.

Results and Discussion

Behavioural observations

Sprague-Dawley rats subjected to chronic constriction injury exhibited abnormal gait, posture, guarding, protective behaviour, and licking of the ipsilateral hind paw within 1–2 days after surgery. The animals were unable to bear weight on the affected limb, which was held close to the body in a characteristic guarding posture. The paw showed marked ventroflexion, with the toes tightly adducted. These abnormal behavioural manifestations persisted even two weeks after the operation.

Effect of i.c.v.-administered NOS inhibitors (L-NG-nitroarginine methyl ester, N-iminoethyl lysine, and 7-nitroindazole) on chronic constriction injury-induced neuropathic Sprague-Dawley rats

Administration of L-NG-nitroarginine methyl ester, N-iminoethyl lysine, and 7-nitroindazole at doses of 50, 100, and 200 µg/rat via the i.c.v. route did not produce any significant changes in pain thresholds of neuropathic Sprague-Dawley rats as assessed by mechanical, radiant heat, and cold stimulation (Figs. 1, 2, and 3).

Effect of i.e.-administered NOS inhibitors (aminoguanidine, L-NG-nitroarginine methyl ester, and 7-nitroindazole) on pain threshold of neuropathic Sprague-Dawley rats

Effect on mechanical stimulation

Intraperitoneal administration of aminoguanidine at doses of 100 and 300 mg/kg significantly increased the pain threshold to mechanical stimulation in neuropathic Sprague-Dawley rats from 3 to 7 h of observation (Fig. 4A), whereas the 30 mg/kg dose produced no change. Similarly, L-NG-nitroarginine methyl ester at doses of 3 and 10 mg/kg did not affect mechanical pain thresholds; however, at 30 mg/kg it significantly increased the pain threshold from 1 to 5 h post-administration (Fig. 4B). 7-nitroindazole at 3 mg/kg did not significantly alter pain thresholds, but doses of 10 and 30 mg/kg produced significant increases from 3 to 5 h and from 1 to 7 h of observation, respectively (Fig. 4C).

Effect on radiant heat stimulation

The effects of intraperitoneally administered aminoguanidine, L-NG-nitroarginine methyl ester, and 7-nitroindazole on radiant heat responses in CCI-induced neuropathic Sprague-Dawley rats are summarized in Fig. 5. Aminoguanidine at doses of 100 and 300 mg/kg significantly prolonged reaction times to radiant heat from 1 to 5 h and from 1 to 7 h, respectively (Fig. 5A), whereas the 30 mg/kg dose was ineffective throughout the observation period. L-NG-nitroarginine methyl ester at 3 and 10 mg/kg did not modify pain thresholds, while a dose of 30 mg/kg significantly increased reaction times to radiant heat from 3 to 5 h after administration

(Fig. 5B). Similarly, 7-nitroindazole at 3 mg/kg had no effect on pain thresholds, whereas doses of 10 and 30 mg/kg significantly increased reaction times at 3 h post-administration (Fig. 5C).

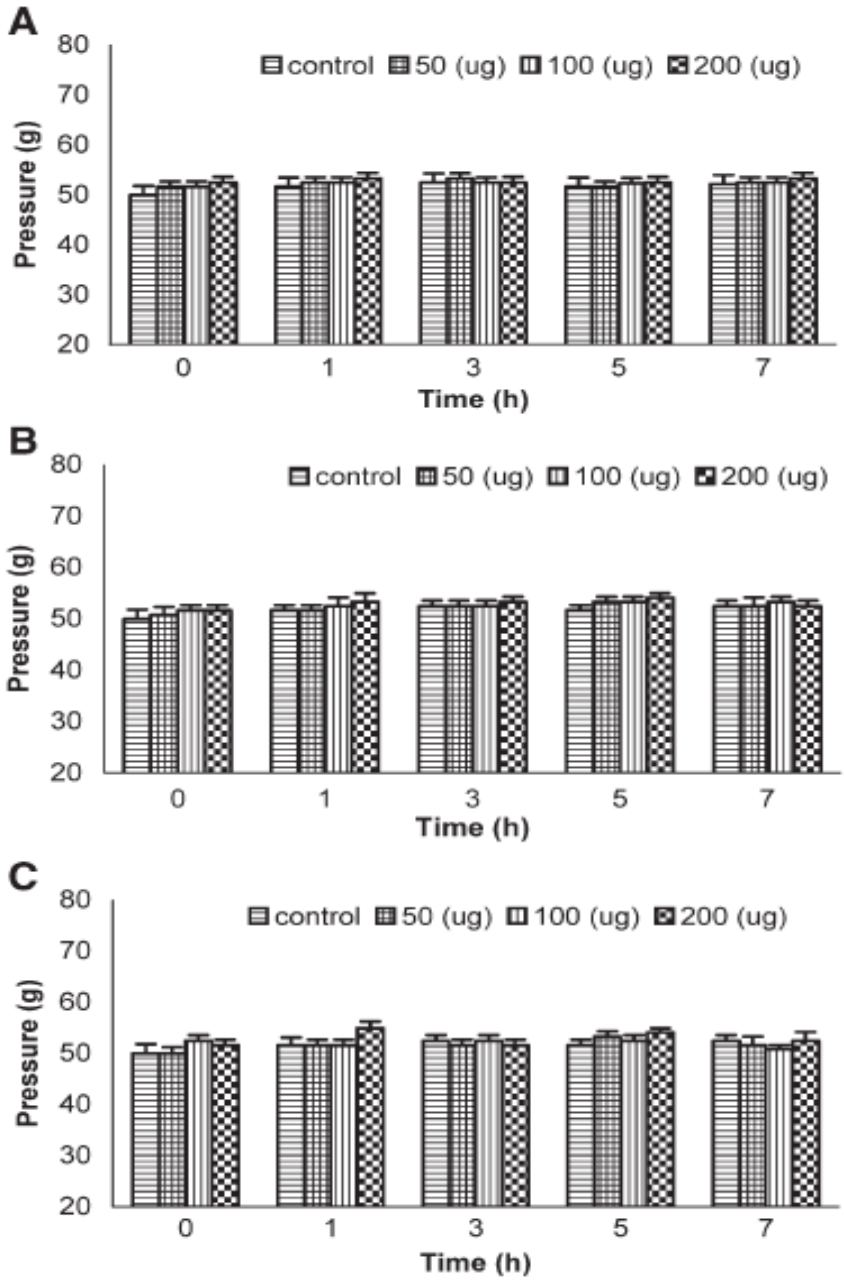

Fig. 1. Effects of i. c. v. administered L-NG-nitroarginine methyl ester (A), N-iminoethyl lysine (B) and 7-nitroindazole (C) on pain produced by mechanical stimulation of hind limb of chronic constriction injury-induced neuropathic rats. Pressure was recorded 1h after i. c. v. administration of drugs. The vertical lines at the top of the bars represent the S.E.M. n=6. ANOVA was employed to compare control with the treatment groups.

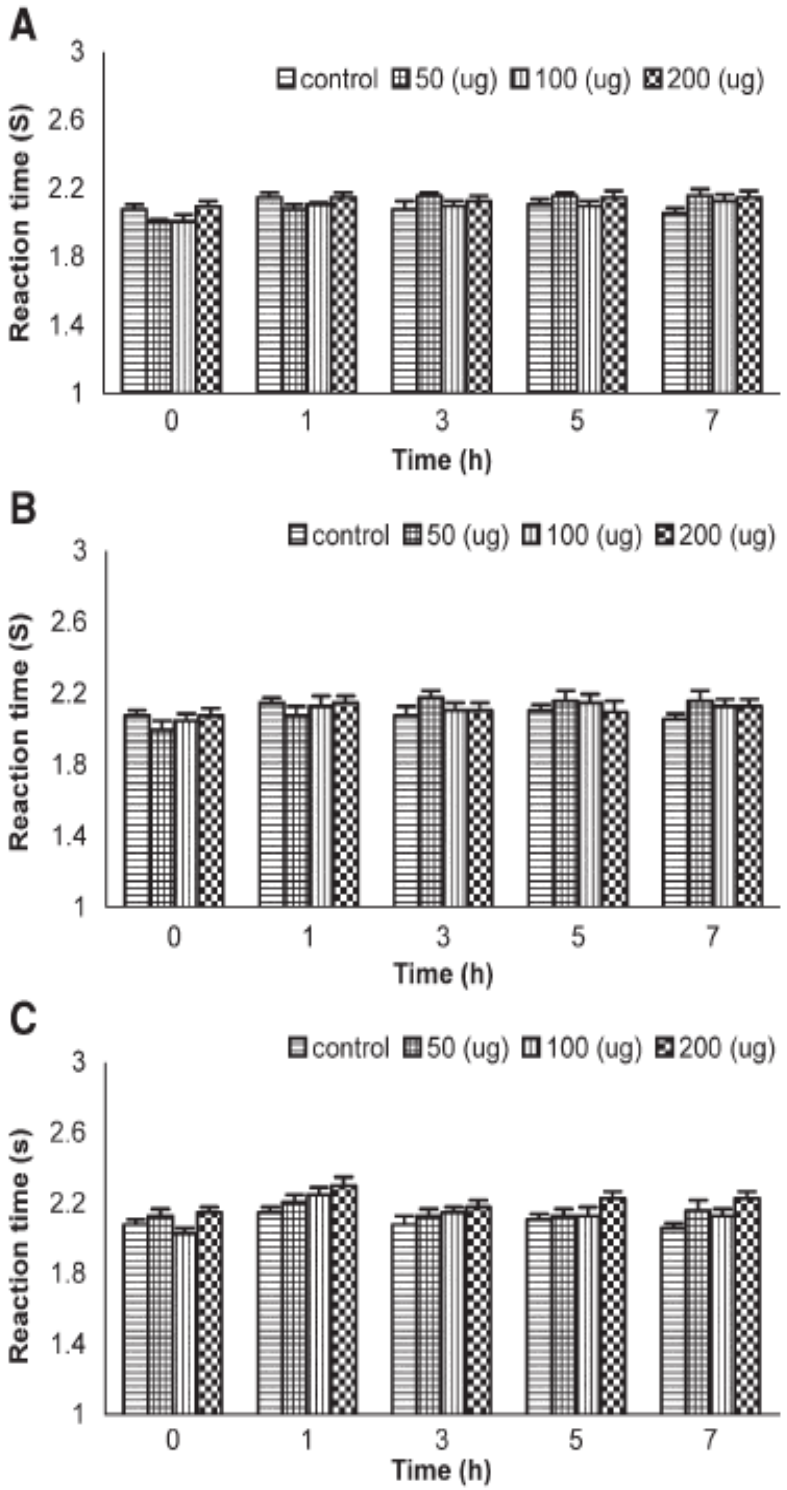

Fig. 2. Effects of i. c. v. administered L-NG-nitroarginine methyl ester (A), N-iminoethyl lysine (B) and 7-nitroindazole (C) on pain produced by radiant heat stimulation of hind limb of chronic constriction injury induced neuropathic rats. Reaction time was recorded 1h after i. c. v. administration of drugs. The vertical lines at the top of the bars represent the S.E.M. n=6. ANOVA was employed to compare control with the treatment groups.

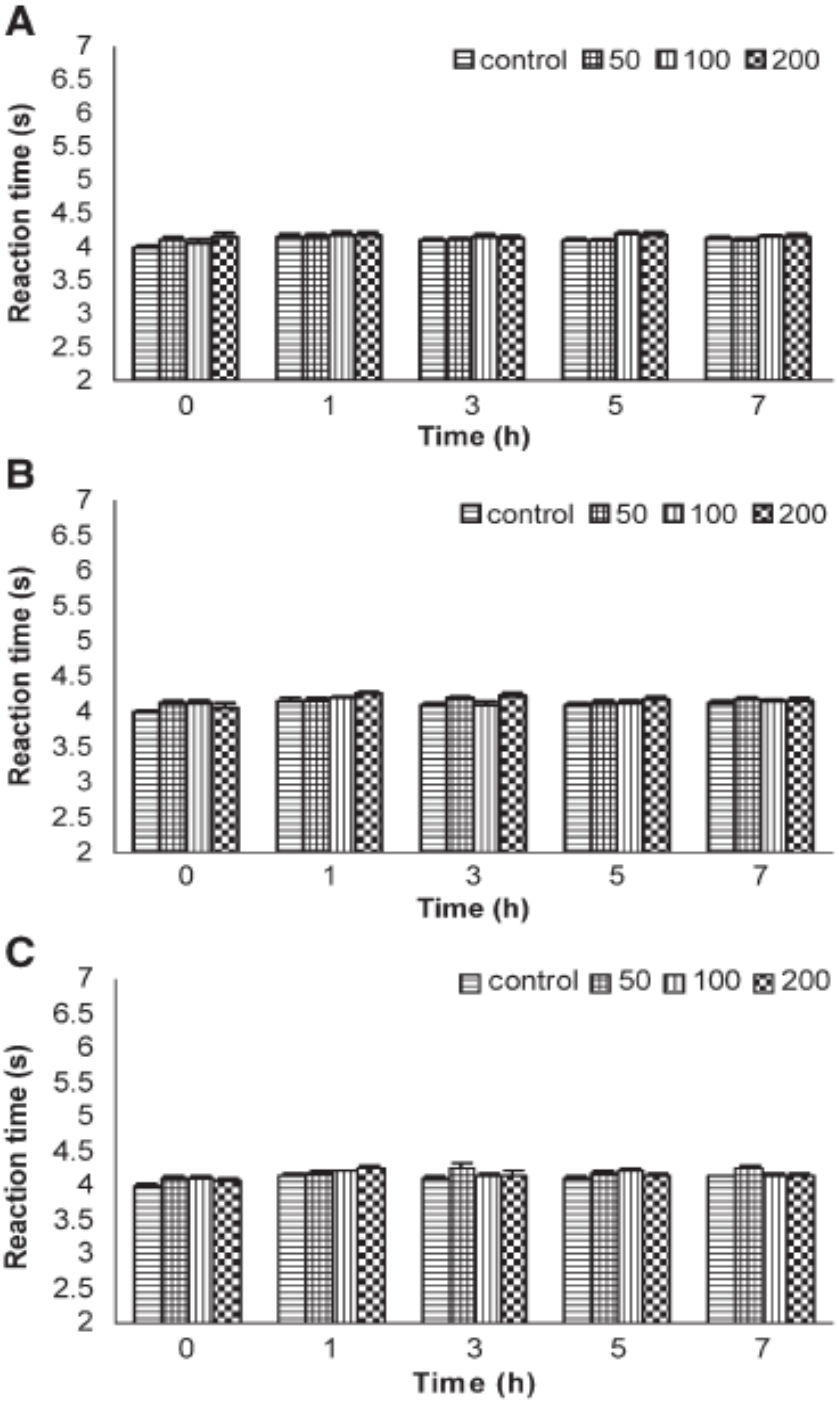

Fig.3 Effects of i.c.v. administered L-NG-nitroarginine methyl ester (A), Niminoethyl lysine (B) and 7-nitroindazole (C) on pain produced by cold allodynia of hind limb of chronic constriction injury-induced neuropathic rats. Reaction time was recorded 1h after i. c. v. administration of drugs. The vertical lines at the top of the bars represent the S.E.M. n=6. ANOVA was employed to compare control with the treatment groups.

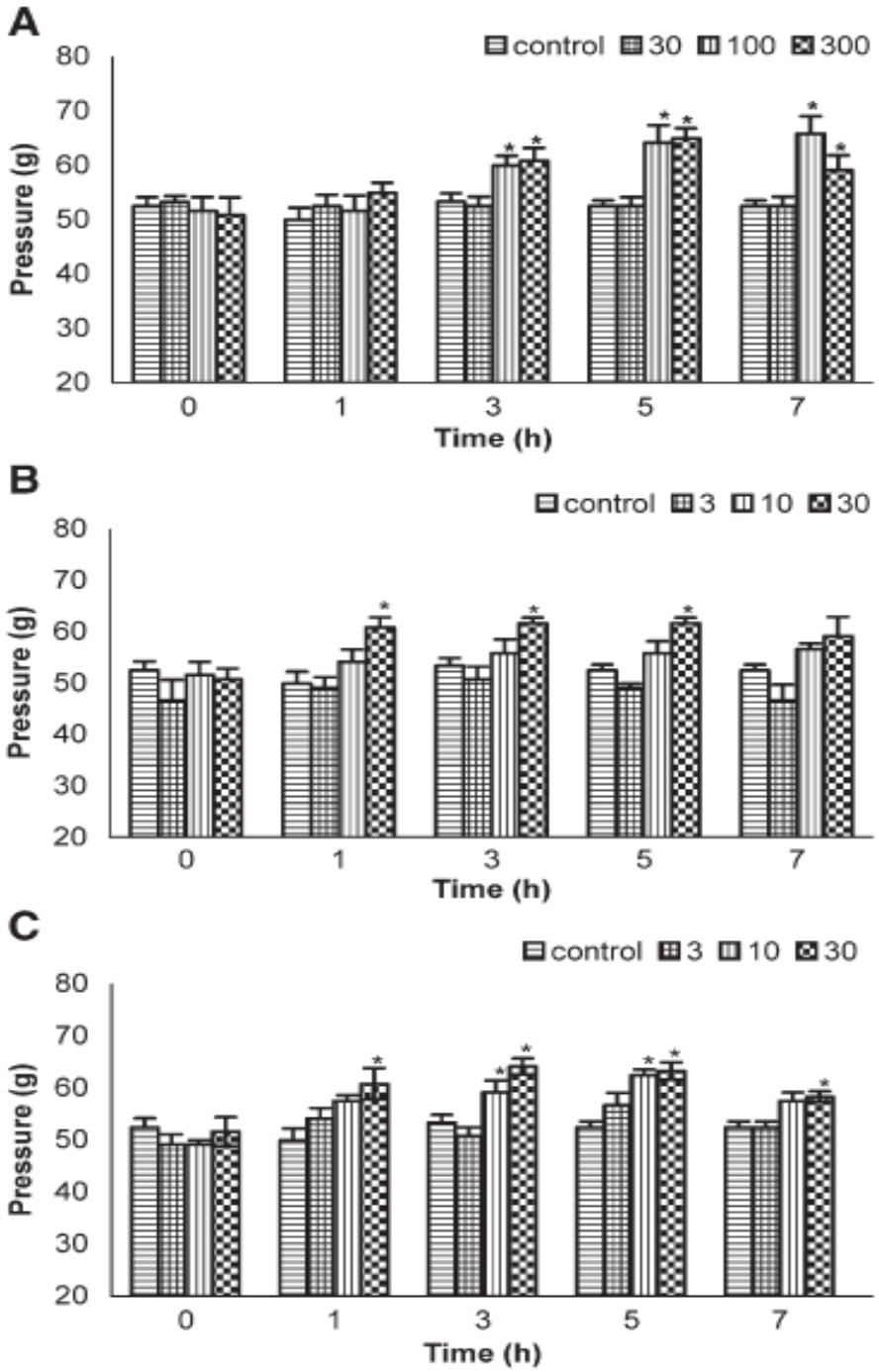

Fig.4 Effect of intraperitoneally administered aminoguanidine (A), L-NGnitroarginine methyl ester (B) and 7-nitroindazole (C) on pain produced by mechanical stimulation of hind limb of chronic constriction injury-induced neuropathic rats. Pressure was recorded 1h after i. p. administration of drugs. The vertical lines at the top of the bars represent the S.E.M. n=6. *pb0.05 compared with control (ANOVA).

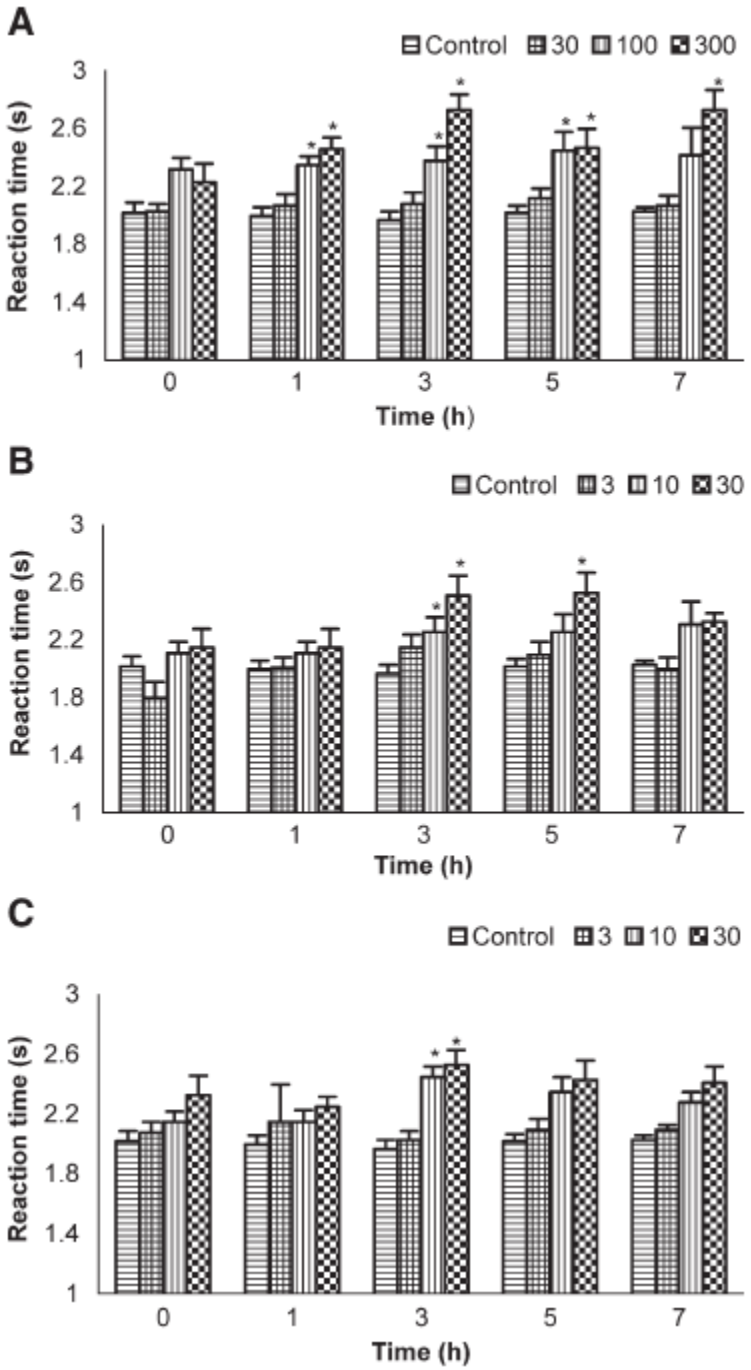

Fig.5 Effect of intraperitoneally administered aminoguanidine (A), L-NGnitroarginine methyl ester (B) and 7-nitroindazole (C) on pain produced by radiant heat stimulation of hind limb of chronic constriction injury-induced neuropathic rats. Reaction time was recorded 1h after i. p. administration of drugs. The vertical lines at the top of the bars represent the S.E.M. n=6. *pb0.05 compared with control (ANOVA).

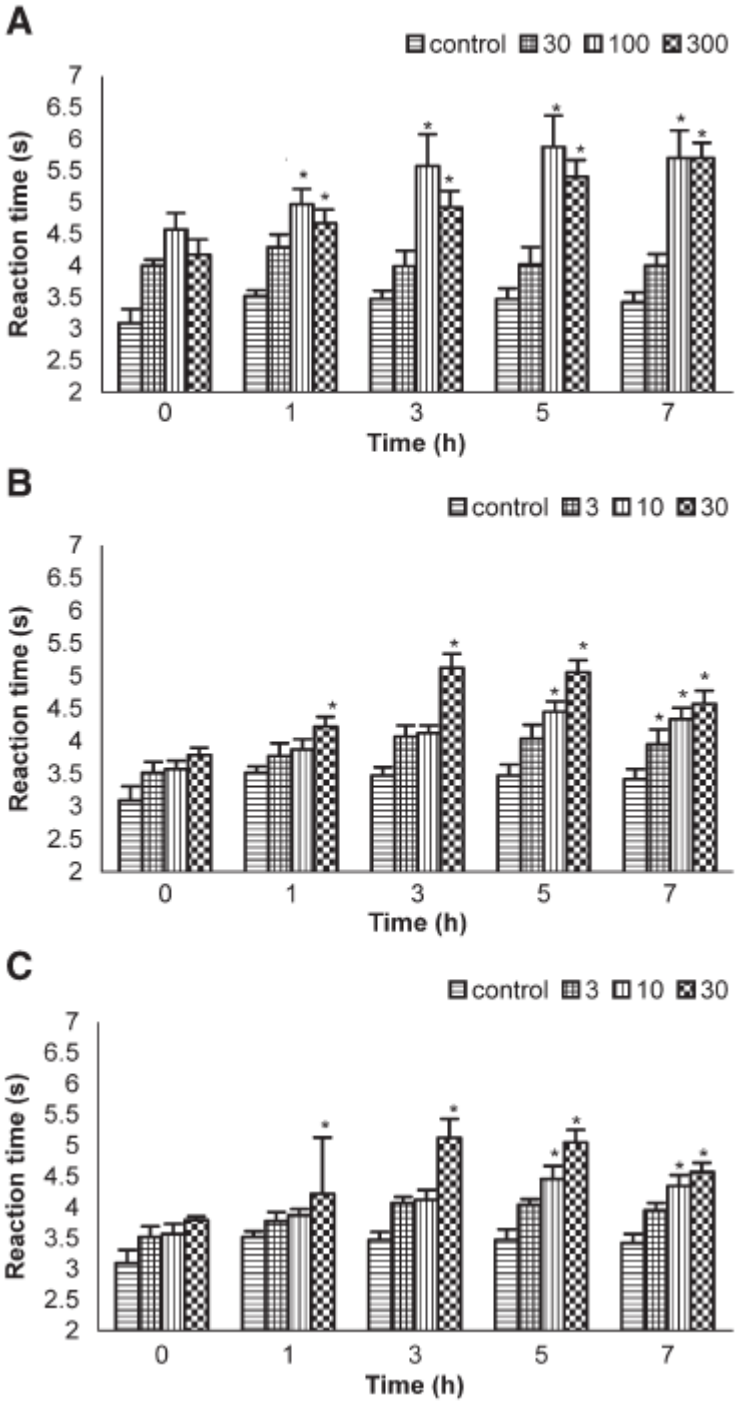

Fig.6 Effect of intraperitoneally administered aminoguanidine (A), L-NGnitroarginine methyl ester (B) and 7-nitroindazole (C) on pain produced by cold allodynia of hind limb of chronic constriction injury-induced neuropathic rats. Reaction time was recorded 1h after i. p. administration of drugs. The vertical lines at the top of the bars represent the S.E.M. n=6. *pb0.05 compared with control (ANOVA).

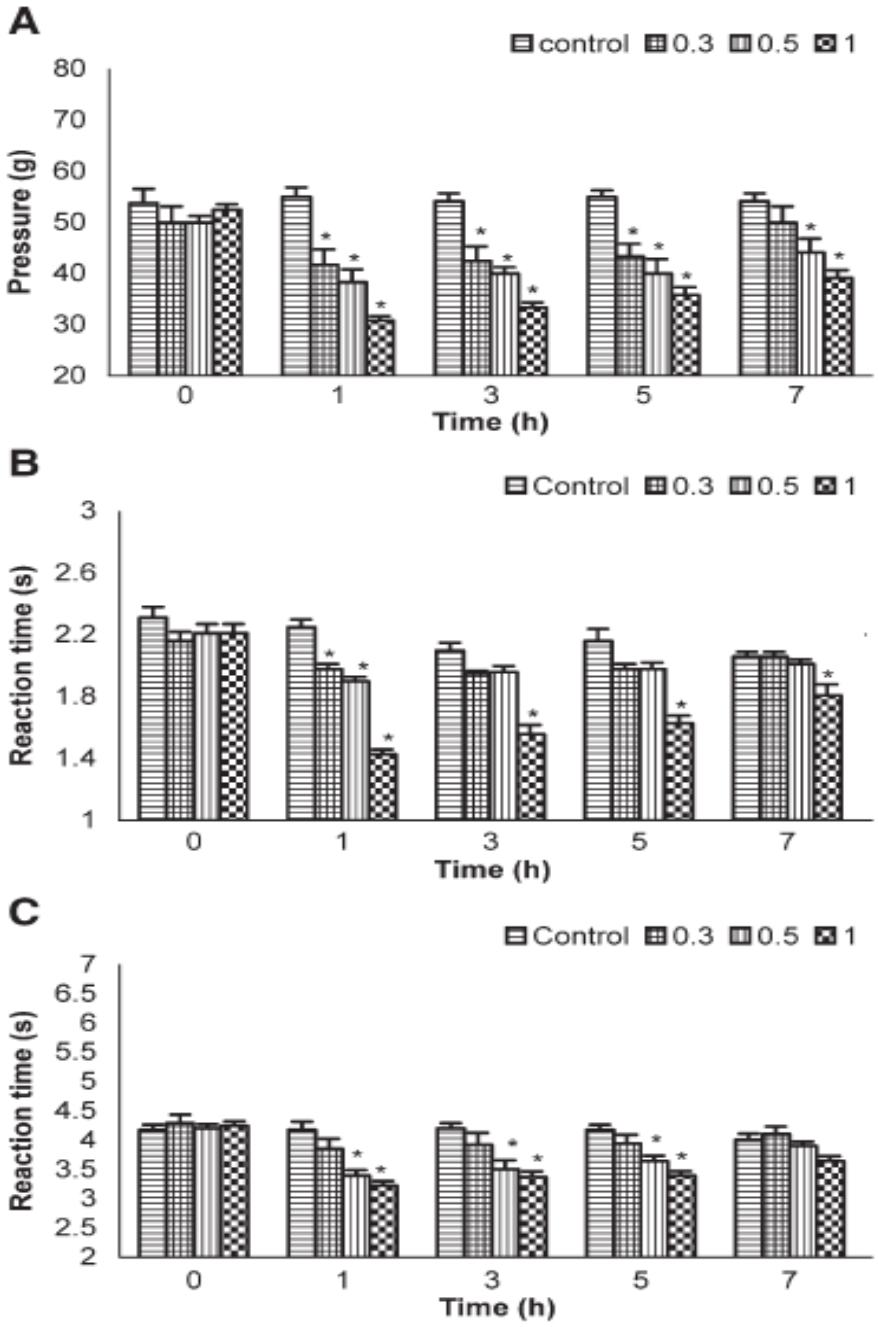

Fig.7 Effect of intraperitoneally administered L-arginine on pain produced by mechanical stimulation (A), radiant heat stimulation (B) and cold allodynia (C) of hind limb of chronic constriction injury-induced neuropathic rats. Pressure/ reaction time was recorded 1h after i. p. administration of drugs. The vertical lines at the top of the bars represent the S.E.M. n=6. *pb0.05 compared with control (ANOVA).

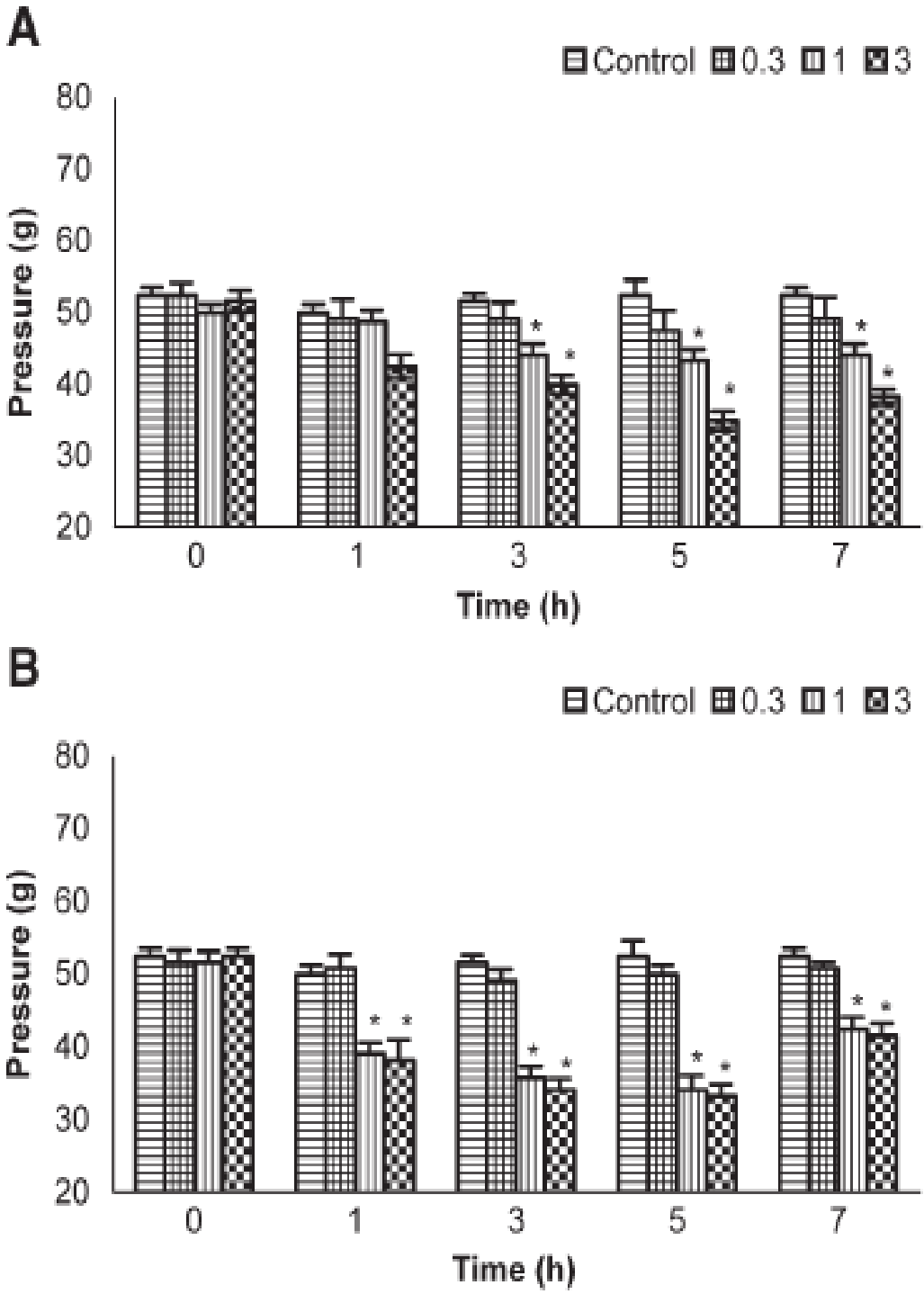

Fig.8 Effect of intraperitoneally administered sodium nitroprusside (A) and Snitroso-N-acetyl penicillamine (B) on pain produced by mechanical stimulation of hind limb of chronic constriction injury-induced neuropathic rats. Pressure was recorded 1h after i. p. administration of drugs. The vertical lines at the top of the bars represent the S.E.M. n=6. *pb0.05 compared with control (ANOVA).

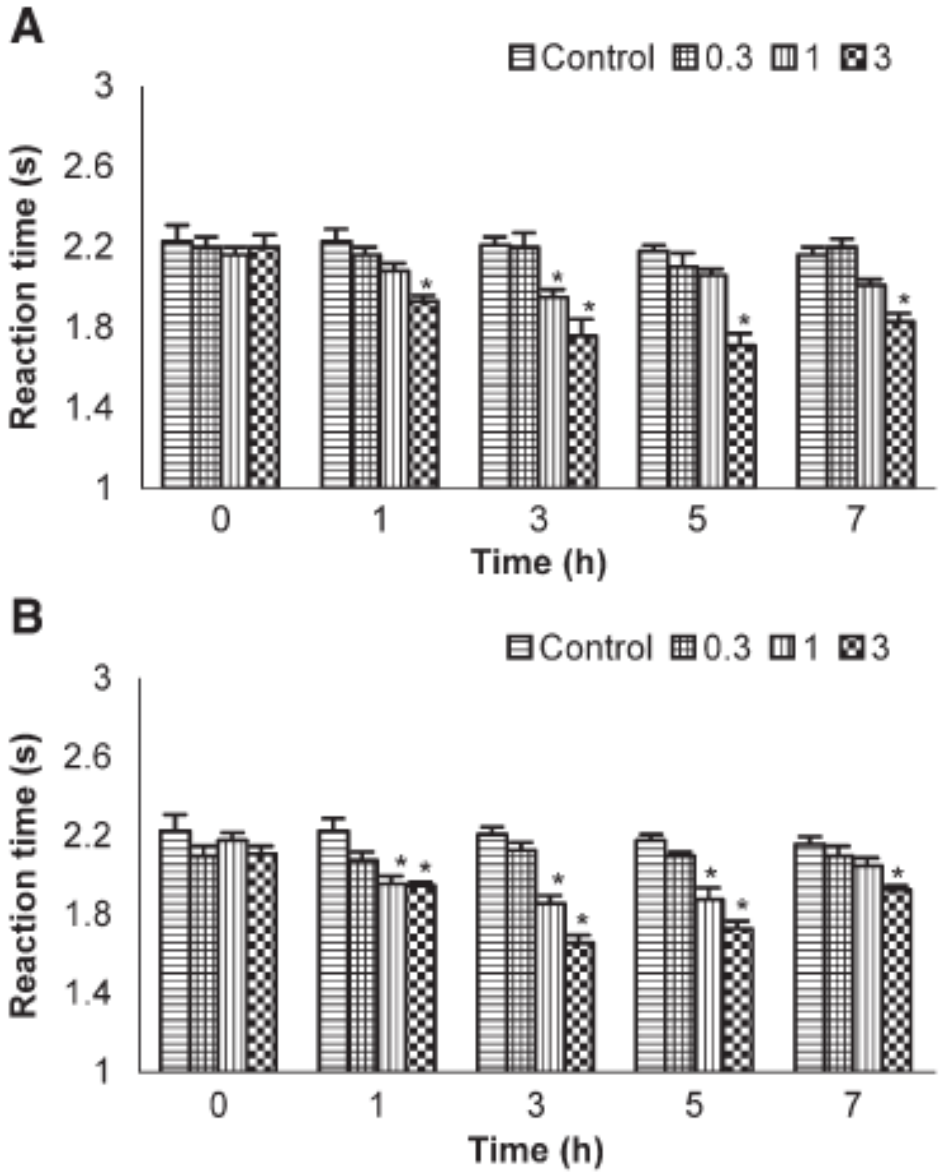

Fig.9 Effect of intraperitoneally administered sodium nitroprusside (A) and Snitroso-N-acetyl penicillamine (B) on pain produced by radiant heat stimulation of hind limb of chronic constriction injury-induced neuropathic rats. Reaction time was recorded 1h after i. p. administration of drugs. The vertical lines at the top of the bars represent the S.E.M. n=6. *pb0.05 compared with control (ANOVA).

Effect on cold allodynia

Aminoguanidine at 30 mg/kg did not influence pain thresholds to cold stimulation; however, doses of 100 and 300 mg/kg significantly increased reaction times from 1 to 7 h of observation (Fig. 6A). L-NG-nitroarginine methyl ester at 3 mg/kg significantly increased reaction times to cold stimuli at 7 h, while doses of 10 and 30 mg/kg enhanced reaction times from 5 to 7 h and from 1 to 7 h, respectively (Fig. 6B). Administration of 7-nitroindazole at 3 mg/kg did not significantly alter reaction times. At 10 mg/kg, it significantly increased reaction times from 5 to 7 h (Fig. 6C), whereas at 30 mg/kg it reduced pain responses from 1 to 7 h of observation.

Effect of i.p.-administered NO precursor L-arginine on pain threshold in chronic constriction injury-induced neuropathic Sprague-Dawley rats

Effect on mechanical stimulation

L-arginine administered at doses of 0.3, 0.5, and 1.0 mg/kg enhanced hyperalgesia in neuropathic Sprague-Dawley rats, with peak effects observed at 1 h following administration; hyperalgesia gradually declined up to 7 h of observation (Fig. 7A).

Effect on radiant heat stimulation

Intraperitoneal administration of L-arginine at doses of 0.3, 0.5, and 1.0 mg/kg produced a significant increase in hyperalgesia to radiant heat. Maximum effects occurred at 1 h post-administration and diminished gradually up to 7 h (Fig. 7B).

Effect on cold allodynia

At doses of 0.5 and 1.0 mg/kg, L-arginine reduced pain thresholds to cold stimulation. Peak hyperalgesia responses were observed at 1 h and declined progressively over the 7 h observation period (Fig. 7C).

Effect of NO donors SNP and SNAP-on pain threshold in chronic constriction injury-induced neuropathic Sprague-Dawley rats

Effect on mechanical stimulation

Sodium nitroprusside and S-nitroso-N-acetyl penicillamine at 0.3 mg/kg did not affect mechanical pain thresholds. However, sodium nitroprusside at doses of 1.0 and 3.0 mg/kg significantly decreased pain thresholds from 3 to 7 h after administration, with maximal effects at 5 h (Fig. 8A). Similarly, S-nitroso-N-acetyl penicillamine at doses of 1.0 and 3.0 mg/kg enhanced hyperalgesia up to 7 h, with peak effects observed at 5 h (Fig. 8B).

Effect on radiant heat stimulation

Intraperitoneal administration of sodium nitroprusside at 0.3 mg/kg did not influence pain withdrawal latencies to radiant heat. At 1.0 mg/kg, sodium nitroprusside significantly reduced pain thresholds at 3 h, while a dose of 3.0 mg/kg produced a marked decrease in withdrawal latencies, with peak effects at 5 h (Fig. 9A). S-nitroso-N-acetyl penicillamine at 0.3 mg/kg was ineffective, whereas doses of 1.0 and 3.0 mg/kg significantly reduced paw withdrawal latencies to radiant heat, with maximum effects observed at 3 h (Fig. 9B).

Effect on cold allodynia

Intraperitoneal administration of sodium nitroprusside and S-nitroso-N-acetyl penicillamine at doses of 0.3, 1.0, and 3.0 mg/kg did not produce any significant changes in cold pain behaviour in neuropathic Sprague-Dawley rats.

Assessment of nitrate and nitrite in the nerves of chronic constriction injury-induced neuropathic Sprague-Dawley rats

Serum nitrate and nitrite concentrations were 49.00 ± 2.38 and 48.33 ± 2.03 µ mol/ml in naïve and CCI-induced neuropathic Sprague-Dawley rats, respectively, showing no significant difference. Similarly, nitrate and nitrite levels in the sciatic nerve did not differ significantly between naïve control Sprague-Dawley rats (56.16 ± 2.26 µ mol/g) and the contralateral sham-operated nerves of CCI Sprague-Dawley rats (60.00 ± 1.36 µmol/g). In contrast, a significant increase in nitrate and nitrite levels was observed in the ligated sciatic nerve (84.16 ± 2.66 µ mol/g) compared with both sham-operated (60.00 ± 1.36 µ

mol/g) and naïve control nerves (56.16 ± 2.20 μ mol/g).

Several experimental models of painful neuropathy have been developed in Sprague-Dawley rats over recent years to investigate the mechanisms involved in the development and maintenance of allodynia and to evaluate the effects of different therapeutic interventions (Kim and Chung, 1992; Kim et al., 1997). Among these models, chronic constriction injury (CCI) of the sciatic nerve in Sprague-Dawley rats is widely used because it produces consistent and persistent tactile allodynia resembling the clinical features observed in patients with neuropathic pain (Bennett and Xie, 1988). In the present study, Sprague-Dawley rats exhibited a marked reduction in thermal, mechanical, and cold thresholds in the hind limb subjected to sciatic nerve constriction, indicating an allodynic state.

Sodium nitroprusside, administered at a dose of 3.0 mg/kg, significantly decreased pain withdrawal latencies, with a peak effect observed at 5 h, as shown in Fig. 9A. S-nitroso-N-acetylpenicillamine at a dose of 0.3 mg/kg did not influence paw withdrawal latencies to radiant heat stimulation. However, administration of S-nitroso-N-acetylpenicillamine at doses of 1.0 and 3.0 mg/kg significantly reduced paw withdrawal duration to radiant heat, with maximal effects observed at 3 h (Fig. 9B).

Effect on cold allodynia

Intraperitoneal administration of sodium nitroprusside and S-nitroso-N-acetyl penicillamine at doses of 0.3, 1.0, and 3.0 mg/kg did not produce any significant alteration in cold pain behaviour in neuropathic Sprague-Dawley rats. Neuropathic pain is not a uniform condition but represents a heterogeneous group of disorders that differ in etiology, anatomical location, and symptomatology (Sindrup and Jensen, 1999; Woolf and Mannion, 1999). The symptoms often do not correlate strictly with either the cause or the anatomical site of injury (Koltzenburg, 1995; Jensen, 1996). Because neuropathic pain frequently responds poorly to conventional treatments (Arner and Meyerson, 1988; Ossipov et al., 1995), considerable attention has been directed toward understanding its underlying mechanisms and developing alternative therapeutic strategies. The role of nitric oxide (NO) at supraspinal sites in neuropathic pain remains unclear, although several studies suggest that spinal NO has pronociceptive properties (Terenghi et al., 1993; Yang et al., 1996). In contrast, spinal NO has also been implicated in morphine-induced antinociception (Kolesnikov et al., 1997; Song et al., 1998). Since the contribution of central NO to the maintenance of neuropathic pain is not fully understood, one objective of the present investigation was to compare the effects of intracerebroventricular (i.c.v.) administration of NOS inhibitors with those of systemic administration on mechanical, thermal, and cold allodynia in CCI-induced neuropathic Sprague-Dawley rats.

To examine the pathophysiological role of NO in neuropathy, four experimental approaches were adopted: (i) evaluation of the effects of NOS inhibitors on neuropathic pain perception, (ii) assessment of the effects of an NO precursor on neuropathic pain, (iii) investigation of the effects of NO donors on neuropathic pain, and (iv) indirect estimation of NOS activity through measurement of nitrate and nitrite levels in the affected nerve.

Alterations in NOS immunoreactivity in lumbar dorsal root ganglia and spinal cord of Sprague-Dawley rats and monkeys have been reported following peripheral axotomy (Zhang et al., 1993). In a peripheral neuropathy model produced by ligation of the

left L5 and L6 nerve roots, increased NOS activity was observed in the ipsilateral L5 and L6 dorsal root ganglia following surgery, with no changes in adjacent ganglia associated with uninjured nerves. These findings suggest that localized changes in NOS activity are relevant to the initiation and/or persistence of altered pain behaviour (Choi et al., 1996). Enhanced NO production, in particular, appears to contribute to neuropathic pain-related behaviours following peripheral nerve injury. Accordingly, peripheral nerve section has been shown to induce up-regulation of NOS activity in ipsilateral dorsal root ganglion neurons (Steel et al., 1994; Verge et al., 1994).

Peripheral nerve injury is also associated with localized up-regulation of inducible NOS (iNOS) in macrophages and Schwann cells, followed by increased NO release. NO contributes to the peripheral nerve injury response by increasing blood flow within the injured nerve trunk (Levy et al., 1999). The expression of iNOS and the resulting local NO production may exert either neurotoxic or neuroprotective effects (Sinz et al., 1999). It has further been demonstrated that spinal cord ligation in Sprague-Dawley rats results in marked up-regulation of NOS, particularly in the ipsilateral gray matter, supporting a role for NO in the pathophysiology of chronic nerve ligation (Cizkova et al., 2002). Because NOS activity is enhanced in CCI-induced neuropathy, increased NO production leads to elevated levels of its metabolites, nitrate and nitrite. Measurement of nitrate and nitrite therefore provides a simple indirect index of NOS activity.

In the present study, nitrate and nitrite levels were estimated in naïve Sprague-Dawley rats and in Sprague-Dawley rats subjected to CCI. A significant increase in nitrate and nitrite concentrations was observed in the sciatic nerves of CCI-induced neuropathic Sprague-Dawley rats compared with naïve and sham-operated animals, whereas serum levels remained unchanged. This indicates that localized NO production within the nerve is critical for the maintenance of pain following nerve injury. Elevated nitrate and nitrite levels have also been reported in the cerebellum and brainstem of neuropathic Sprague-Dawley rats (Onal et al., 2003), further supporting up-regulation of NOS activity in neuropathic pain, particularly within the sciatic nerve.

Based on evidence of increased NO production and NOS expression following nerve injury, the present investigation employed L-NG-nitroarginine methyl ester, N-iminoethyl lysine, and 7-nitroindazole administered via the i.c.v. route, as well as aminoguanidine, L-NG-nitroarginine methyl ester, and 7-nitroindazole administered intraperitoneally, to assess their effects on pain perception in CCI-induced neuropathic Sprague-Dawley rats. Intracerebroventricular administration of L-NG-nitroarginine methyl ester, N-iminoethyl lysine, and 7-nitroindazole did not alter pain perception, possibly because the inhibitors were administered ipsilaterally to the ligated sciatic nerve. Inhibition of NOS in the ipsilateral somatosensory cortex may have left sufficient residual NOS activity to sustain nociceptive processing (Salter et al., 1996). This observation is consistent with reports indicating that many NO-dependent behaviours are affected only when NOS inhibition exceeds 50% (Kapas et al., 1994; Salter et al., 1995). Nevertheless, antihyperalgesic effects of L-NG-nitroarginine methyl ester have been reported when administered contralaterally to the injured nerve (Salter et al., 1996). Other studies have also demonstrated antinociceptive effects of i.c.v. administered NOS inhibitors in different pain models (Przewlocka et al., 1994; Sarma, 2000). Differences in nociceptive and neuropathic mechanisms may further explain the lack of

effect observed with ipsilateral i.c.v. administration in the present study.

In contrast, intraperitoneal administration of aminoguanidine, L-NG-nitroarginine methyl ester, and 7-nitroindazole significantly attenuated pain behaviour in CCI-induced neuropathic Sprague-Dawley rats. Aminoguanidine, a selective iNOS inhibitor, has not been extensively studied in rodent neuropathic pain models. In the present study, aminoguanidine reduced mechanical hyperalgesia and also delayed hyperalgesic responses to radiant heat and cold stimuli. Since peripheral nerve injury is associated with iNOS up-regulation in macrophages and Schwann cells at and distal to the injury site (Levy et al., 1999), inhibition of the iNOS–NO pathway by aminoguanidine likely accounts for the observed reduction in hyperalgesia.

L-NG-nitroarginine methyl ester, a non-selective NOS inhibitor, and 7-nitroindazole, a selective neuronal NOS inhibitor (Hao and Xu, 1996), also alleviated chronic allodynia-like symptoms when administered systemically. Similar antihyperalgesic effects of L-NG-nitroarginine methyl ester have been reported in CCI Sprague-Dawley rats when delivered directly to the injury site via osmotic pumps (Thomas et al., 1996) and in other neuropathic pain models (Meller et al., 1992; Hao et al., 1994; Yamamoto and Shimoyama, 1995). Likewise, 7-nitroindazole has shown antinociceptive activity in several neuropathy models (Allawi et al., 1994; Hao and Xu, 1996). These findings support the involvement of NO in both the development and maintenance of hyperalgesia in neuropathic conditions and suggest that local NO production at the site of nerve injury plays a more prominent role than central NO production.

L-arginine, a semi-essential basic amino acid (pH 5–6.5; Boger and Bode-Boger, 2001), serves as the substrate for NO synthesis by NOS isoforms and can induce hyperalgesia through NMDA receptor activation (Kitto et al., 1992). NOS activity is inhibited by L-arginine analogues such as NG-monomethyl-L-arginine and NG-nitro-L-arginine, and this inhibition can be reversed by excess L-arginine. Intrathecal administration of L-arginine has been shown to induce autotomy behaviour, which is attenuated by L-NG-nitroarginine methyl ester (Niedbala et al., 1995).

In the present study, intraperitoneal administration of L-arginine to neuropathic Sprague-Dawley rats produced marked hyperalgesia in response to mechanical, radiant heat, and cold stimuli, with peak effects observed at 1 h post-administration. These findings confirm the hyperalgesic role of NO in neuropathic pain and are consistent with previous observations in other nociceptive models (Moore et al., 1993; Honore et al., 1995; Takano et al., 1998).

NO donors, including sodium nitroprusside and S-nitroso-N-acetyl penicillamine, were administered intraperitoneally at doses of 0.3, 1.0, and 3.0 mg/kg, and pain perception was assessed using mechanical, radiant heat, and cold stimuli up to 7 h post-administration. Both donors produced hyperalgesia in neuropathic Sprague-Dawley rats, with peak effects occurring between 3 and 5 h. Mechanical and radiant heat hyperalgesia were evident, whereas responses to cold stimuli were not significantly affected. Although S-nitroso-N-acetyl penicillamine has previously been shown to induce hyperalgesia in paw pressure and tail flick tests (Przewlocka et al., 1994), the present study represents the first demonstration of its hyperalgesic effects in a chronic pain model. These findings are further supported by studies showing that systemic administration of another NO donor, nitroglycerine, significantly reduced tail flick latency in formalin-induced pain models (Tassorelli et al., 2003).

CONCLUSION

In conclusion, the present findings indicate that enhanced local production of nitric oxide plays a significant role in the maintenance of pain in neuropathic Sprague-Dawley rats and that this pain is reduced by inhibition of nitric oxide synthase. Hyperalgesia is further exacerbated by the nitric oxide precursor L-arginine and nitric oxide donors such as sodium nitroprusside and S-nitroso-N-acetyl penicillamine. These initial efforts to modulate the L-arginine–NO pathway suggest the therapeutic potential of such interventions in the management of neuropathic pain.

References

Kapas, L., Fang, J., Krueger, J.M., 1994. Inhibition of nitric oxide synthesis suppresses sleep in rats. *Brain Res.* 664, 189–196.

Kim, K.J., Yoon, Y.W., Chung, J.M., 1997. Comparative evaluation of three rodent models of neuropathic pain. *Exp. Brain Res.* 113, 200–206.

Kim, S.H., Chung, J.M., 1992. An experimental rat model of peripheral neuropathy produced by segmental spinal nerve ligation. *Pain* 50, 355–363.

Kitto, K.F., Haley, J.E., Wilcox, G.L., 1992. Role of nitric oxide in spinally mediated hyperalgesia in mice. *Neurosci. Lett.* 148, 1–5.

Kolesnikov, Y.A., Pan, Y.X., Babey, A.M., Jain, S., Wilson, R., Pasternak, G.W., 1997. Functional differentiation of two neuronal nitric oxide synthase isoforms using antisense mapping: evidence for opposing nitric oxide actions on morphine analgesia and tolerance. *Proc. Natl. Acad. Sci. U.S.A.* 94, 8220–8225.

Koltzenburg, M., 1995. Stability and plasticity of nociceptor function and their relation to evoked and ongoing pain. *Semin. Neurosci.* 7, 199–210.

Levy, D., Hoke, A., Zochodne, D.W., 1999. Local inducible nitric oxide synthase expression in an animal model of neuropathic pain. *Neurosci. Lett.* 260, 207–209.

Malmberg, A.B., Basbaum, A.I., 1998. Partial sciatic nerve injury in mice as a model of neuropathic pain: behavioral and neuroanatomical correlates. *Pain* 76, 215–222.

Meller, S.T., Pechman, P.S., Gebhart, G.F., Maves, T.J., 1992. Nitric oxide mediates thermal hyperalgesia in a rat model of neuropathic pain. *Neuroscience* 50, 7–10.

Moore, P.K., Wallace, P., Gaffen, Z., Hart, S.L., Babbedge, R.C., 1993. Characterization of the novel nitric oxide synthase inhibitor 7-nitroindazole and related indazoles: antinociceptive and cardiovascular actions. *Br. J. Pharmacol.* 110, 219–224.

Niedbala, B., Sanchez, A., Feria, M., 1995. Nitric oxide mediates neuropathic pain behavior in peripherally denervated rats. *Neurosci. Lett.* 188, 57–60.

Onal, A., Delen, Y., Ulker, S., Soykan, N., 2003. Agmatine attenuates neuropathic pain in rats: possible involvement of nitric oxide and noradrenergic mechanisms in the brainstem and cerebellum. *Life Sci.* 73, 413–428.

Ossipov, M.H., Lopez, Y., Nicholas, M.L., Bian, D., Porreca, F., 1995. Prevention of loss of spinal morphine antinociceptive efficacy in nerve-ligated rats by reducing spinal afferent input. *Neurosci. Lett.* 199, 87–90.

Przewlocka, B., Machelska, H., Przewlocki, R., 1994. Involvement of the nitric oxide pathway in central nociceptive processing in rats. *Regul. Pept.* I, S75–

S76.

Randall, L.O., Selitto, J., 1957. A method for assessing analgesic activity in inflamed tissue. *Arch. Int. Pharmacodyn.* 111, 209–219.

Rowbotham, M.C., 1994. Management of post-herpetic neuralgia using opioids and local anesthetics. *Ann. Neurol.* 35, S46–S49.

Salter, M., Duffy, C., Garthwaite, J., Strijbos, P.J.L.M., 1995. Regional and hemispheric differences in brain nitric oxide synthase inhibition following intracerebroventricular administration of Nω-nitro-L-arginine and L-NAME. *Neuropharmacology* 34, 639–649.

Salter, M., Strijbos, P.J.L.M., Neale, S., Duffy, C., Follenfant, R.L., Garthwaite, J., 1996. Requirement of the nitric oxide–cyclic GMP pathway for nociceptive signaling at specific somatosensory loci. *Neuroscience* 73, 649–655.

Sarma, J., Role of central nitrinergic system in experimentally induced inflammation and nociception in rats. Ph.D. Thesis, Indian Veterinary Research Institute, Izatnagar, U.P., India.

Sastry, K.V.H., Moudgal, R.P., Mohan, J., Tyagi, J.S., Rao, G.S., 2002. Spectrophotometric determination of serum nitrite and nitrate using a copper–cadmium alloy. *Anal. Biochem.* 306, 79–82.

Seltzer, Z., Dubner, R., Shir, Y., 1990. A novel behavioral model of neuropathic pain produced in rats by partial sciatic nerve injury. *Pain* 43, 205–218.

Sindrup, S.H., Jensen, T.S., 1999. Effectiveness of pharmacological treatments for neuropathic pain: an update with mechanism-based considerations. *Pain* 83, 389–400.

Sinz, E.H., Kochanek, P.M., Dixon, C.E., Clark, R.S., Carcillo, J.A., Schiding, J.K., Chen, M., Nisniewski, S.R., Carlos, T.M., Williams, D., DeKosky, S.T., Watkins, S.C., Marion, D.W., Billiar, T.R., 1999. Inducible nitric oxide synthase as an endogenous neuroprotectant following traumatic brain injury in rats and mice. *J. Clin. Invest.* 104, 647–656.

Song, H.K., Pan, H.L., Eisenach, J.C., 1998. Spinal nitric oxide mediates antinociception induced by intravenous morphine. *Anesthesiology* 89, 215–221.

Steel, J.H., Terenghi, G., Chung, J.M., Na, H.S., Carlton, S.M., Polak, J.M., 1994. Increased nitric oxide synthase immunoreactivity in rat dorsal root ganglia in a neuropathic pain model. *Neurosci. Lett.* 169, 81–84.

Takano, H., Lim, H.B., Miyabara, Y., Ichinose, T., Yoshikawa, T., Sagai, M., 1998. Oral L-arginine administration enhances allergen-induced airway inflammation and interleukin-5 expression in mice. *J. Pharmacol. Exp. Ther.* 286, 767–771.

Tal, M., 1996. A novel antioxidant alleviates heat hyperalgesia in rats with experimental peripheral neuropathy. *NeuroReport* 7, 1382–1384.

Tanelian, D.L., Brose, W.G., 1991. Neuropathic pain relief by use-dependent sodium channel blockers: lidocaine, carbamazepine, and mexiletine. *Anesthesiology* 74, 949–951.

Tassorelli, C., Greco, R., Wang, D., Sandrini, G., Nappi, G., 2003. Nitroglycerin-induced hyperalgesia in rats: a time-course study. *Eur. J. Pharmacol.* 464, 159–162.

Terenghi, G., Riveros-Moreno, V., Hudson, L.D., Ibrahim, N.B.N., Polak, J.M., 1993. Immunohistochemical localization of nitric oxide synthase in spinal cord and dorsal root ganglia of

humans and rats. *J. Neurol. Sci.* 118, 34–37.

Thomas, D.A., Ren, K., Besse, D., Ruda, N.A., Dubner, R., 1996. Application of nitric oxide synthase inhibitor Nω-nitro-L-arginine methyl ester to injured nerve attenuates neuropathy-induced thermal hyperalgesia in rats. *Neurosci. Lett.* 210, 124–126.

Tsuda, M., Shigemoto-Mogami, Y., Koizumi, S., Mizokoshi, A., Kohsaka, S., Salter, M.W., Inoue, K., 2003. Induction of P2X4 receptors in spinal microglia mediates tactile allodynia following nerve injury. *Nature* 424, 778–783.

Verge, V.M.K., Zhang, X., Xu, X.J., Wiesenfeld-Hallin, Z., Hokfelt, T., 1994. Marked increase in nitric oxide synthase mRNA in rat dorsal root ganglia following peripheral axotomy: in situ hybridization and functional analyses. *Proc. Natl. Acad. Sci. U.S.A.* 89, 11617–11621.

Verster, F., Robinson, C.A., Hengveld, C.A., Bush, E.S., 1971. A freehand cerebroventricular injection technique for unanesthetized rats. *Life Sci.* 10, 1395–1402.

Woolf, C.J., Mannion, R.J., 1999. Neuropathic pain: etiology, symptoms, mechanisms, and management. *Lancet* 353, 1959–1964.

Yamamoto, T., Shimoyama, N., 1995. Role of nitric oxide in the development of thermal hyperesthesia induced by sciatic nerve constriction injury in rats. *Anesthesiology* 82, 1266–1273.

Yang, L.C., Marsala, M., Yaksh, T.L., 1996. Time course of spinal amino acids, citrulline, and PGE_2 release following carrageenan/kaolin-induced knee joint inflammation: a chronic microdialysis study. *Pain* 67, 345–354.

Zhang, X., Verge, V., Wiesenfeld-Hallin, Z., Ju, G., Bredt, D., Snyder, S.H., Hokfelt, T., 1993. Nitric oxide synthase-like immunoreactivity in lumbar dorsal root ganglia and spinal cord of rats and monkeys and the effects of peripheral axotomy. *J. Comp. Neurol.* 335, 563–575.

How to cite this article:

Naik, A. K. 2020. Nitric Oxide and its Modulators in Chronic Constriction Injury-Induced Neuropathic Pain in Sprague-Dawley Rats. *Int.J.Curr.Microbiol.App.Sci.* 9(10): 4077-4097.
doi: https://doi.org/10.20546/ijcmas.2020.910.468