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Protective effect of retigabine in rat neuropathic pain model: Influence of nitric oxide modulators

2.1. Introduction

Neuropathic pain (NP) is defined as “pain arising because of a lesion or disease of the somatosensory system” (Jensen et al. 2011). NP is characterized by spontaneous pain, hyperalgesia (an augmented response to a stimulus which is normally painful) and allodynia (pain as a result of a stimulus which does not normally provoke pain) etc. The molecular mechanisms underlying neuropathic pain are not yet clearly understood. However, various mechanisms such as damage to the sensory neurons resulting in adaptive changes in the density, distribution and functional activities of several voltage & ligand gated ion channels, receptors and enzymes in the damaged DRG including spinal neurons (Rasband et al. 2001; Waxman and Zamponi 2014) have been proposed. These mechanistic changes in part have been demonstrated to be responsible for the state of hyperexcitability (Abdulla and Smith 2001; Abdulla and Smith 2002).

Opening of potassium channels plays an essential role in regulating resting membrane potential through hyperpolarization of cell membrane resulting decreased excitability. There are five different types of voltage gated K+ channel subunits (Kv7.1-7.5) which are the members of KCNQ/M channels family (Brown and Passmore 2009). A slowly activating (tens of milliseconds), low threshold (activate at about -60mV), non inactivating, steady voltage dependent outward current (M current) generated by these channels exerts a suppressing effect on recurring or burst-firing as well as on general excitability of the neurons (Brown and Passmore 2009; Delmas and Brown 2005). Drug interventions which are aimed at these Kv7 channels have been recently shown to be effective in different types of inflammatory (Xu et al. 2010; Nielsen et al. 2004), visceral (Hirano et al. 2007) and neuropathic conditions (Zheng et al. 2013; Nodera et al. 2011).

Besides, role of ROS has also been well documented in persistent chronic pain (Kim et al. 2004; Naik et al. 2006). Further, free radical scavengers have been
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shown to be effective in ameliorating NP. Indeed, retigabine, a potent Kv7 channel opener has been shown to produce antioxidant as well as neuroprotective effect in different in vitro studies (Boscia et al. 2006; Seyfried et al. 2000). NMDA receptor activation after persistent peripheral neuronal discharge results in an increased intracellular calcium release which further activates protein kinase C that increases NOS and NO production. NO thus produced in turn activates NO-cGMP pathways facilitating central sensitization in spinal dorsal horn (Schmidtko 2015). In periphery, increased NO leads to an increased prostaglandin (PG) production especially PGE$_2$ and PGI$_2$ via mechanisms that are not clear causing peripheral sensitization and pain perception (Mollace et al. 2005). Ori and his group proposed the potential role of M current and its modification by nitric oxide modulator in trigeminal ganglion neurons regulation (Ooi et al. 2013). However, the relationship between KCNQ/M channels and NO pathway in neuropathic painful condition and their influence on various behavioral manifestations have not been studied so far.

Hence, the current study has been intended to investigate the potential of retigabine (KCNQ/M channel modulator) and its interaction with NO modulators in L5/L6 spinal nerve ligation induced NP.

2.2. Materials and Methods

2.2.1. Animals

Male Sprague Dawley (SD) rats (180–220 g) bred in Central Animal House, Panjab University, Chandigarh were utilized in this study. Animals were habituated to experimental conditions before commencement of actual tests. They were maintained on 12-h light/dark cycle with food and water ad libitum. All

Figure 2.1 Experimental protocol
the tests were performed in between 9:00am and 3:00pm. Ethical clearance from IAEC of Panjab University was obtained prior to the start of the experiment (IAEC/411/UIPS-41, 11/9/13) and performed as per the Indian National Science Academy guidelines for the use and care of experimental animals. All the behavioral studies were assessed by a subject who is blinded to the treatment.

### 2.2.2. Spinal nerve ligation

Spinal nerve ligation was performed as per modified method of Chung (Chung et al. 2004). Briefly, under chloral hydrate (350mg/kg) anesthesia, individual animal has been placed in prone position & beginning from left L4 to till S2 level spinous process is freed from the adhered muscle tissue. L6 transverse process was identified and part of it is removed cautiously in order to expose L4-L6 branches of spinal nerves. A 6-0 silk thread was passed beneath the L5 & L6 spinal nerves and tightly ligated distal to the DRG and just before the formation of common sciatic nerve. Following complete haemostasis, the wound was sutured. The procedure of the sham treated animals was similar to the ligated group except spinal nerves ligation. Sterile conditions were maintained throughout the surgical procedures.

### 2.2.3. Drug and treatment schedule

Study protocol includes eleven treatment groups (n=6). Retigabine was obtained from Mylan laboratories (India), suspended in 1% v/v Tween 80 and administered orally (p.o.) as per body weight (5 ml/kg). L-arginine and L-NAME [N(G)-nitro-L-arginine methyl ester] (Sigma Chemicals, St. Louis, USA) were prepared with saline (pH 7.4) and administered intraperitoneally (i.p.) 30 minutes prior to retigabine treatment.

The study was carried out in two phases; first phase consists of the effect of retigabine and second phase involves its interaction with NO modulators. Various treatment groups employed in the present protocol have been depicted in Table 2.1.
Table 2.1 Treatment groups

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment group</th>
<th>Treatment (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Naive</td>
<td>Healthy animals (No treatment given)</td>
</tr>
<tr>
<td>2</td>
<td>Sham</td>
<td>surgery performed, vehicle administered</td>
</tr>
<tr>
<td>3</td>
<td>SNL</td>
<td>L5/L6 spinal nerves were ligated</td>
</tr>
<tr>
<td>4</td>
<td>SNL+ Ret (5)</td>
<td>SNL + Retigabine (5 mg/kg, p.o.)</td>
</tr>
<tr>
<td>5</td>
<td>SNL+ Ret (10)</td>
<td>SNL + Retigabine (10 mg/kg, p.o.)</td>
</tr>
<tr>
<td>6</td>
<td>SNL+ L-NAME (10)</td>
<td>SNL + L-NAME (10 mg/kg, i.p.)</td>
</tr>
<tr>
<td>7</td>
<td>SNL+ L-NAME (10) + Ret (5)</td>
<td>SNL + L-NAME (10 mg/kg, i.p.) + Retigabine (5 mg/kg, p.o.)</td>
</tr>
<tr>
<td>8</td>
<td>SNL+ L-arginine (100)</td>
<td>SNL + L-arginine (100 mg/kg, i.p.)</td>
</tr>
<tr>
<td>9</td>
<td>SNL+ L-arginine (100) + Ret (5)</td>
<td>SNL + L-arginine (100 mg/kg, i.p.) + Retigabine (5 mg/kg, p.o.)</td>
</tr>
<tr>
<td>10</td>
<td>SNL+ GP (100)</td>
<td>SNL + Gabapentin (100 mg/kg, i.p.)</td>
</tr>
<tr>
<td>11</td>
<td>Ret (10) per se</td>
<td>Retigabine (10 mg/kg, p.o.) treatment in sham treated animals</td>
</tr>
</tbody>
</table>

Each group received respective treatment daily at 10:00 am, for 28 days starting from the day after SNL. Drugs and their doses were selected as per reported literature (Dost et al. 2004; Patil et al. 2006). All the behavioral tests were performed after 1 hr of drug administration.

2.2.4. Behavioral estimations

2.2.4.1. Mechanical alldynia

Refer to chapter 1 (1.2.4.3)

2.2.4.2. Mechanical hyperalgesia

Refer to chapter 1 (1.2.4.4)
2.2.4.3. Cold allodynia

The cold allodynia was assessed by application of a 200 μL of acetone onto the plantar region of rat paw. During the acetone application touching with the skin was avoided. The total time that animal spent on lifting, shaking or licking against acetone treatment that was recorded for 2 min immediately after acetone application (Choi et al. 1994).

2.2.5. Biochemical estimations

2.2.5.1 Dissection and homogenization1

Immediately after behavioral estimations on day 28, animals were sacrificed by cervical dislocation & sciatic nerve (starting from the point of appearance from the spinal cord to its trifurcation, so that it covers the ligated portion along with proximal and distal ends) was dissected out and stored at -20°C for biochemical assays. For the biochemical tests, 10% (w/v) tissue homogenates were prepared using 0.1 M phosphate buffer (pH 7.4). The obtained homogenates were centrifuged at 10,000 × g at 4 °C for 15 min. Supernatants obtained after centrifugation were separated & used for different biochemical estimations.

2.2.5.2. Measurement of endogenous antioxidant parameters

2.2.5.2.1. Estimation of lipid peroxidation

Refer to chapter 1 (1.2.7.1.1).

2.2.5.2.2. Estimation of nitrite

Nitrite concentration which is a measure of amount of NO produced in the supernatant, was quantified with a colorimetric assay involving Greiss reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid) as described by (Green et al. 1982). Supernatant and Greiss reagent were mixed in equal quantities, which then kept at room temperature for 10 min in the dark. Absorbance was determined at 540 nm using Perkin Elmer Lambda 20 spectrophotometer (Norwalk, CT, USA). The amount of nitrite in the test sample was determined by a sodium nitrite standard curve and expressed as micromole per milligram of protein.

2.2.5.2.3. GSH estimation

Refer to chapter 1 (1.2.7.1.2).

2.2.5.2.4. Catalase estimation

Refer to chapter 1 (1.2.7.1.4)
2.2.5.2.5. Protein estimation

Refer to chapter 1 (1.2.7.1.5).

2.2.6. Statistical analysis

Refer to chapter 1 (1.2.8)

2.3. Results

2.3.1. Effect of retigabine and its interactions with nitric oxide modulators on mechanical allodynia in spinal nerve ligation induced neuropathic pain

No significant difference on paw withdrawal threshold was observed between sham and naive group. Ligation of L5/L6 spinal nerves of the control group significantly ($p < 0.001$) reduced paw withdrawal threshold as compared to sham treated animals. Retigabine (10 mg/kg, p.o.) treatment significantly ($p < 0.01$) raised paw withdrawal threshold from day 14 as compared to control SNL group (Fig. 2.2A).

![Graph showing the effect of retigabine on mechanical allodynia in spinal nerve ligation induced neuropathic pain.](image)

**Figure 2.2A** Effect of retigabine on mechanical allodynia in spinal nerve ligation induced neuropathic pain. Data were expressed as mean ± S.E.M. $^a p < 0.05$ compared to sham group; $^b p < 0.05$ compared to SNL group; $^c p < 0.05$ compared to Ret (5); (Two way ANOVA followed by Bonferroni posttests). SNL: spinal nerve ligation, Ret: retigabine.
However, lower dose of retigabine (5 mg/kg, p.o.) significantly improved paw withdrawal threshold starting from day 21 as compared to SNL control group. The protective effect of retigabine (10 mg/kg) was comparable with that of gabapentin (100 mg/kg) treatment in SNL treated animals. However, retigabine (10 mg/kg) per se treatment did not demonstrate any significant effect on paw withdrawal threshold as compared to sham treatment (Fig. 2.2A).

Further, prior administration of L-NAME (10 mg/kg, i.p.) before retigabine (5 mg/kg, p.o.) significantly potentiated their protective effect (raised paw withdrawal threshold) on 21st and 28th day as compared to their effect per se in SNL treated group ($p < 0.05$). Conversely, L-arginine (100 mg/kg, i.p.) pretreatment with retigabine (5 mg/kg, p.o.) significantly ($p < 0.05$) reversed the protective effect (shortened paw withdrawal threshold) of retigabine (5 mg/kg) on 3rd and 4th week in SNL treated group (Fig. 2.2B).

Figure 2.2B Effect of retigabine along with the nitric oxide modulators on mechanical allodynia in spinal nerve ligation induced neuropathic pain. Data were expressed as mean ± S.E.M. $^a p < 0.05$ compared to sham group; $^b p < 0.05$ compared to SNL group; $^c p < 0.05$ compared to Ret (5); $^d p < 0.05$ compared to L-NAME (10); (Two way ANOVA followed by Bonferroni posttests). SNL: spinal nerve ligation, Ret: retigabine, L-Arg: L-arginine.

2.3.2. Effect of retigabine and its interactions with nitric oxide modulators on mechanical hyperalgesia in spinal nerve ligation induced neuropathic pain

No significant difference in mechanical hyperalgesic response (change in paw withdrawal threshold) was observed between naive and sham group animals.
Whereas, SNL significantly ($p < 0.001$) reduced paw withdrawal threshold as compared to sham group. Retigabine (10 mg/kg, p.o.) treatment significantly ($p < 0.05$) improved paw withdrawal threshold from day 7 onwards as compared to SNL control group. However, lower dose of retigabine (5 mg/kg, p.o.) significantly improved paw withdrawal threshold from day 14 onwards as compared to SNL control group. Further, gabapentin (100 mg/kg) treatment significantly increased paw withdrawal threshold from day 14 onwards as compared to SNL control group. However, retigabine (10 mg/kg) per se treatment did not demonstrate any significant effect on mechanical hyperalgesia as compared to sham treatment (Fig. 2.3A).

Further, L-NAME (10 mg/kg, i.p.) pretreatment with retigabine (5 mg/kg, p.o.) significantly potentiated their protective effect (raised paw withdrawal threshold) from day 21 onwards as compared to their effects per se in SNL treated animals ($p < 0.05$). Conversely, L-arginine (100 mg/kg) pretreatment with lower dose of retigabine (5 mg/kg, p.o.) significantly reversed the protective effect of retigabine on day 28 in SNL treated group (Fig. 2.3B).

**Figure 2.3A** Effect of retigabine on mechanical hyperalgesia in spinal nerve ligation induced neuropathic pain. Data were expressed as mean ± S.E.M. $^{a}p$ $< 0.05$ compared to sham group; $^{b}p$ $< 0.05$ compared to SNL group; $^{c}p$ $< 0.05$ compared to Ret (5); (Two way ANOVA followed by Bonferroni posttests). SNL: spinal nerve ligation, Ret: retigabine.
Figure 2.3B Effect of retigabine along with the nitric oxide modulators on mechanical hyperalgesia in spinal nerve ligation induced neuropathic pain. Data were expressed as mean ± S.E.M. a$p < 0.05$ compared to sham group; $p < 0.05$ compared to SNL group; $p < 0.05$ compared to Ret (5); $p < 0.05$ compared to L-NAME (10); (Two way ANOVA followed by Bonferroni posttests). SNL: spinal nerve ligation, Ret: retigabine, L-ARG: L-arginine.

2.3.3. Effect of retigabine and its interactions with nitric oxide modulators on cold allodynia in spinal nerve ligation induced neuropathic pain

There was no significant difference in cold allodynia [paw withdrawal duration (lifting and licking)] was observed between sham and naive group animals. SNL treatment significantly ($p < 0.001$) caused cold allodynia (reduced time latency to paw withdrawal), and produced more licking and lifting response against acetone treatment that persisted throughout the study period as compared to sham group. Retigabine (10 mg/kg, p.o.) treatment significantly reversed cold allodynia (improved time latency to paw withdrawal), reduced licking and lifting from day 7 onwards ($p < 0.05$) as compared to SNL control group. However, treatment with lower dose of retigabine (5 mg/kg, p.o.) significantly attenuated cold allodynia from day 21 onwards as compared to SNL control group. Further, gabapentin (100 mg/kg) treatment delayed time latency to paw withdrawal (lifting and licking) from day 21 onwards as compared to SNL control group. However, retigabine (10 mg/kg) per se treatment did not demonstrate any significant effect on cold allodynia as compared to sham group (Fig. 2.4A).
Fig. 2.4A Effect of retigabine on cold allodynia in spinal nerve ligation induced neuropathic pain. Data were expressed as mean ± S.E.M. \( ^a p < 0.05 \) compared to sham group; \( ^b p < 0.05 \) compared to SNL group; \( ^c p < 0.05 \) compared to Ret (5); (Two way ANOVA followed by Bonferroni posttests). SNL: Spinal nerve ligation, Ret: Retigabine.

Fig. 2.4B Effect of retigabine along with the nitric oxide modulators on cold allodynia in spinal nerve ligation induced neuropathic pain. Data were expressed as mean ± S.E.M. \( ^a p < 0.05 \) compared to sham group; \( ^b p < 0.05 \) compared to SNL group; \( ^c p < 0.05 \) compared to Ret (5); \( ^d p < 0.05 \) compared to L-NAME (10); (Two way ANOVA followed by Bonferroni posttests). SNL: Spinal nerve ligation, Ret: Retigabine, L-ARG: L-arginine.
Further, L-NAME (10 mg/kg, i.p.) pretreatment with retigabine (5 mg/kg, p.o.) significantly potentiated their protective effect (decreased paw withdrawal duration, reduced licking and lifting) from day 14 onwards as compared to their effects per se ($p < 0.05$) in SNL treated animals. Conversely, L-arginine (100 mg/kg, i.p.) pretreatment with lower dose of retigabine (5 mg/kg, p.o.) reversed the protective effect of retigabine from day 14 onwards in SNL treated group (Fig. 2.4B).

### 2.3.5. Effect of retigabine and its interaction with nitric oxide modulators on oxidative damage in spinal nerve ligated rats

No significant effect on oxidative damage parameters was observed between sham and naive group. SNL treatment significantly ($p < 0.001$) increased lipid peroxidation, nitrite concentration, depleted GSH & catalase activity in sciatic nerves as compared to sham group. Treatment with retigabine (5 & 10 mg/kg) and gabapentin (100 mg/kg) for 28 days significantly ($p < 0.05$) attenuated lipid peroxidation, nitrite levels, restored GSH, & catalase activities as compared to SNL control group.

Further, prior administration of L-NAME (10 mg/kg) before retigabine (5 mg/kg, p.o.) significantly ($p < 0.05$) potentiated their protective effect (reduced lipid peroxidation, nitrite levels, restored GSH, & catalase activity) as compared to their effect per se in SNL treated animals. Conversely, pretreatment of L-arginine (100mg/kg) before retigabine (5 mg/kg p.o.) significantly reversed the protective effect of retigabine (5 mg/kg) (increased lipid peroxidation, nitrite concentration, and depleted GSH & catalase activity) in SNL treated animals. However, retigabine (10 mg/kg) per se treatment did not produce any significant effect on oxidative damage parameters as compared to sham group (Table 2.2).
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Table 2.2 Effect of retigabine on oxidative damage (lipid peroxidation, nitrite, catalase and GSH levels) and its interaction with nitric oxide modulators in spinal nerve ligation induced neuropathic pain in rats

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>MDA nM/mg protein Mean ± S.E.M. (% of sham)</th>
<th>Nitrite µM/mg protein Mean ± S.E.M. (% of sham)</th>
<th>Catalase µM of H$_2$O$_2$ decomposed per min/mg protein Mean ± S.E.M. (% of sham)</th>
<th>GSH (nM/mg protein) Mean ± S.E.M (%Sham)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>1.577 ± 0.07 (99.47)</td>
<td>66.67 ± 2.50 (98.77)</td>
<td>2.949 ± 0.24 (100.40)</td>
<td>32.62 ± 0.99 (102.55)</td>
</tr>
<tr>
<td>Sham</td>
<td>1.585 ± 0.05 (100)</td>
<td>67.50 ± 2.08 (100)</td>
<td>2.94 ± 0.34 (100)</td>
<td>31.82 ± 0.53 (100)</td>
</tr>
<tr>
<td>SNL</td>
<td>4.398 ± 0.20a (277.33)</td>
<td>300.83 ± 3.74a (445.68)</td>
<td>0.39 ± 0.05a (13.42)</td>
<td>5.32 ± 0.63a (16.73)</td>
</tr>
<tr>
<td>SNL + Ret (5)</td>
<td>3.690 ± 0.11b (232.77)</td>
<td>234.67 ± 5.16b (347.65)</td>
<td>1.41 ± 0.10b (48.00)</td>
<td>11.79 ± 1.00b (37.05)</td>
</tr>
<tr>
<td>SNL + Ret (10)</td>
<td>2.928 ± 0.14b,c (184.69)</td>
<td>186.67 ± 5.51b (276.54)</td>
<td>2.03 ± 0.11b,c (69.13)</td>
<td>18.10 ± 1.20b,c (56.89)</td>
</tr>
<tr>
<td>SNL + L-NAME (10)</td>
<td>4.083 ± 0.18b,c (257.63)</td>
<td>290.50 ± 9.08b (430.37)</td>
<td>0.47 ± 0.04b,c (15.93)</td>
<td>7.55 ± 0.59b,c (23.74)</td>
</tr>
<tr>
<td>SNL + L-NAME (10) + Ret (5)</td>
<td>2.765 ± 0.16b,c,d (174.46)</td>
<td>202.67 ± 4.77b,c,d (300.25)</td>
<td>2.43 ± 0.12b,c,d (82.87)</td>
<td>17.82 ± 1.43b,c,d (56.01)</td>
</tr>
<tr>
<td>SNL + L-Arg (100)</td>
<td>4.465 ± 0.13b,c (281.67)</td>
<td>310.33 ± 9.34b,c (459.75)</td>
<td>0.43 ± 0.04b,c (14.61)</td>
<td>6.49 ± 0.65b,c (20.39)</td>
</tr>
<tr>
<td>SNL + L-Arg (100) + Ret (5)</td>
<td>4.385 ± 0.13c (276.51)</td>
<td>306.17 ± 9.32c (453.58)</td>
<td>0.95 ± 0.09c (32.40)</td>
<td>5.88 ± 0.76c (18.49)</td>
</tr>
<tr>
<td>SNL + GP (100)</td>
<td>2.224 ± 0.17b,c,e (140.29)</td>
<td>98.00 ± 5.54b,c,e (145.19)</td>
<td>2.57 ± 0.23b,c,e (87.53)</td>
<td>18.39 ± 1.58b,c,e (57.81)</td>
</tr>
<tr>
<td>Sham + Ret (10)</td>
<td>1.560 ± 0.11b,c (98.43)</td>
<td>71.67 ± 3.45b,c (106.17)</td>
<td>0.43 ± 0.04b,c (101.17)</td>
<td>30.23 ± 2.25b,c (95.03)</td>
</tr>
</tbody>
</table>

Data is expressed as mean ± S.E.M; in parenthesis percentage of sham was mentioned. $^a$p < 0.05 compared to sham group; $^b$p < 0.05 compared to SNL group; $^c$p < 0.05 compared to Ret (5); $^d$p < 0.05 compared to L-NAME (10); $^e$p < 0.05 compared to Ret (10); (One way ANOVA followed by Tukey’s test). SNL: spinal nerve ligation, Ret: retigabine.
2.4. Discussion

Injury to peripheral nerve and its branches by transection, crush or ligation resulted in development of spontaneous pain, alldynia and hyperalgesia, well known characteristics of neuropathic pain (Padi and Kulkarni 2008; Sekiguchi et al. 2009). These behavioral alterations vary in duration and magnitude depending on the experiment model or species. These behavioral alterations last up to several weeks. In the present study, SNL (L5/L6 spinal nerve ligation) significantly caused hyperalgesia and alldynia to both mechanical as well as thermal stimuli indicating neuropathic like symptoms. Different animal models have been developed to mimic NP like symptoms analogous to clinical conditions in humans. Few of these models involve loose ligation of a part of the sciatic nerve as in the CCI model (Kumar et al. 2011) or tight ligation of portion of the sciatic nerve as in the partial sciatic nerve ligation model (Rose et al. 2011). In both these models, there exists a considerable degree of variability in tightness of the ligation and the extent of nerve damaged by ligation. Both of these difficulties can be resolved by tight ligation of lumbar L5 and L6 spinal branches of the sciatic nerve. This L5 and L6 ligation model also has an advantage of causing separate injury on nerve branches to enable further manipulations in the neuropathic pain conditions. In the present study, SNL resulted a significant alterations in behavioral (mechanical alldynia, mechanical hyperalgesia, cold alldynia and heat hyperalgesia) and biochemical (lipid peroxidation, nitrite concentration, GSH, and catalase) parameters in sciatic nerves supporting neuropathic like symptoms. Our observations are in accordance with the previous experimental studies demonstrating similar behavioral, biochemical alterations against SNL (Sekiguchi et al. 2009; Chung et al. 2004; Xu et al. 2010; Yowtak et al. 2011).

Retigabine is a structural analogue of flupirtine, a non opiate. It has a broad spectrum of anticonvulsant activity. Its analgesic activity has been demonstrated in various experimental models of chronic pain. Retigabine has been reported to reduce mechanical hyperalgesia in CCI model and SNI, but failed to show any significant effect on alldynic response against vonfrey stimulation in both the experimental models (Blackburn-Munro and Jensen 2003). In the current study, retigabine treatment significantly attenuated behavioral alterations such as mechanical alldynia, mechanical hyperalgesia, and cold alldynia suggesting its
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ameliorative effect against SNL induced neuropathic like symptoms. Exact reasons for these protections are not yet known. Differences in the site (spinal and sciatic level) and extent of injury produced could be responsible for the differences observed.

Role of ROS has been well explored in the pathophysiology of NP (Kim et al. 2004; Yowtak et al. 2011). In the present study, SNL significantly caused oxidative stress as evidenced by an increased lipid peroxidation, nitrite levels, depletion of GSH, & catalase in sciatic nerves. 28 day treatment with retigabine significantly attenuated oxidative stress in sciatic nerve as evidenced by reduced lipid peroxidation, nitrite levels, restored GSH, and catalase in sciatic nerves suggesting its antioxidant like effect. Different antioxidants, free radical scavenging substances, peroxynitrite-decomposition catalysts have been shown to reduce the mechanical alldynia in different models of NP (Kim et al. 2004; Kumar et al. 2007; Arora et al. 2008). In support, retigabine has also been demonstrated to reduce ROS in rat pheochromocytoma PC 12 cells (Seyfried et al. 2000) as well as in organotypic hippocampal slice cultures (Boscia et al. 2006). Retigabine has been shown to restore glutathione levels and decreased reactive oxygen species production caused by L-glutamate toxicity in rat pheochromocytoma PC 12 cells (Seyfried et al. 2000).

In support, present study has also demonstrated that retigabine to restore GSH, and catalase levels suggesting its role in promoting antioxidant defense. Therefore, it is likely that an antioxidant defense property of retigabine could be partially responsible for its protective effect as one of the mechanism. Even though, the potential antioxidant effect through reduction in reactive oxygen species has been studied in vitro, to the best of our knowledge, this is the first study to demonstrate antioxidant like effect of retigabine in vivo. Nonetheless, the precise mechanism by which ROS contribute to the development and maintenance of hypersensitivity in NP is not clearly known. Additional studies are required to elucidate the exact mechanism to prove the antioxidant like effect of retigabine.

Peripheral nerve injury resulted an up regulation of NOS activity in the DRG and lumbar dorsal horn have been observed in different NP models (Choi et al. 2012; Kim et al. 2011; Luo et al. 1999). Increased NO reacts with superoxide radicals to form peroxynitrile, which further takes part in the phosphorylation of NMDA receptors in dorsal horn leading to central sensitization in different neuropathic pain conditions
(Kwak et al. 2014). In trigeminal ganglion neurons administration of S-nitroso-N-acetyl-DL-penicillamine, a NO donor, inhibited M current which is reversed by NOS inhibitors and scavenger of NO, suggesting that NO mediated alteration of M current in these neurons (Ooi et al. 2013). However, modulation of neuronal M current through redox modulatory site has also been proposed (Ooi et al. 2013). Consistent with this, \( \text{H}_2\text{O}_2 \) resulted in an increase in M current whereas NO reduced M current. Further, these modulations are attributed to exert through triple cysteine catalytic site present in DRG neurons. Strict control of NO environment around this catalytic site has been proposed to be further responsible for change in M current. In line with these studies, SNL resulted an increased NO production as indicated by rise in nitrite levels in the sciatic nerves of the present study. It seems that rise in NO might further affect M current that is responsible for protective effect of retigabine (Fig 2.6). Further, L-NAME pretreatment with retigabine significantly potentiated their protective effect. However, L-arginine pretreatment with retigabine significantly reversed its protective effect signifying the NO mediated alteration of retigabine effect. This implies the role of NO mediated alterations in the protective effects of retigabine in SNL induced oxidative stress parameters. However, earlier studies involving linopiridine (KCNQ channel blocker) in combination with retigabine in neuropathic pain models has been studied to demonstrate the mechanism of action of retigabine (Dost et al. 2004). It has been proposed that an antiallodynic response of retigabine is mediated through the opening of KCNQ channels and was antagonized by linopiridine in neuropathic pain. But the down stream process that effects the retigabine actions through the opening of KCNQ channels has not been clearly understood. The results of the present study demonstrate that possible involvement of NO mediated alterations in the protective effect of retigabine in NP (Fig 2.6). In summary, the present study highlights the role of retigabine in SNL induced behavioral and biochemical alterations. Further, the protective effect of retigabine could be at least in part due to its antioxidant like effects in addition to its effect on KCNQ channel. The interaction of retigabine along with NO modulators suggests the possible involvement of NO mediated alterations in the protective effect of retigabine in SNL induced NP. On the other hand, the complex pathophysiological process does not rule out the involvement of other possible mechanisms of neuropathic pain. Nonetheless, further studies are needed to demonstrate the exact mechanism of retigabine in neuropathic conditions.
Figure 2.6 Possible targets of action of retigabine in SNL induced neuropathic pain in rats.