Dexmedetomidine alleviated neuropathic pain in dorsal root ganglion neurons by inhibition of anaerobic glycolysis activity and enhancement of ROS tolerance

Running title: Analgesic effect and mechanism of action of dexmedetomidine on neuropathic pain

Peibin Liu1, *, Tufeng Chen2,4, Fang Tan1, Jingling Tian1, Lei Zheng1, Yingqing Deng1, Jiaxin Chen1, Xinjin Chi1*

1Department of Anaesthesiology, The Seventh Affiliated Hospital of Sun Yat-Sen University, Shenzhen 518071, P.R China

2Department of Gastrointestinal Surgery, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou 510530, P.R China

* Correspondence: Dr Xinjin Chi, Department of Anaesthesiology, The Seventh Affiliated Hospital of Sun Yat-Sen University, 628 Zhenyuan Road, Guangming District, Shenzhen 518071, P.R China. Email: chixinjin@yeah.net. Tel: (+86)075581206682

† These authors contributed equally to this study.

Author Contributions:

Xinjin Chi conceived and designed the experiments; Peibin Liu and Tufeng Chen performed the experiments; Tufeng Chen, Fang Tan and Jingling Tian analyzed the data; Lei Zheng, Yingqing Deng and Jiaxin Chen contributed reagents/materials/analysis tools; Xinjin Chi and Peibin Liu wrote the paper.
Disclosure of conflict of interest: No conflict of interest

Funding: The study was partly supported by the Natural Science Foundation of China (No. 81873644), Science and Technology Planning Project of Guangdong Province, China (Grant No. 2017A020215074)
Abstract

Neuropathic pain is a kind of chronic pain that is triggered or caused primarily by damage to the nervous system and neurological dysfunction. It’s known that dexmedetomidine is a new type of highly selective alpha2-adrenoceptor agonist with sedation, anti-anxiety, analgesic and other effects. However, the function and mechanism of dexmedetomidine on neuropathic pain are not clear. Rat DRG neurons were isolated and identified using immunofluorescence assay. Following treatment with H2O2, dexmedetomidine or ROS inhibitor (NAC), the apoptosis and ROS levels were examined by flow cytometry; apoptosis-related proteins and anaerobic glycolysis-related proteins were determined by western blot assay; glucose consumption, pyruvic acid, lactic acid and ATP/ADP ratios were also measured. The results revealed that dexmedetomidine inhibited H2O2-induced apoptosis and reactive oxygen species (ROS) in rat DRG neurons and in addition, dexmedetomidine downregulated the expression levels of anaerobic glycolysis-related proteins, significantly reduced glucose, pyruvic acid and lactic acid levels. It also increased the ATP/ADP ratio in H2O2-treated rat dorsal root ganglion (DRG) neurons. Moreover, we also demonstrated that ROS inhibitor (NAC) also inhibited H2O2-induced apoptosis and anaerobic glycolysis in rat DRG neurons. In conclusion, dexmedetomidine suppressed H2O2-induced apoptosis and anaerobic glycolysis activity by inhibiting ROS, in rat DRG neurons. Therefore, dexmedetomidine might play a pivotal role in neuropathic pain by the inhibition of ROS.

Keywords: dexmedetomidine, neuropathic pain, dorsal root ganglion neurons, anaerobic glycolysis, apoptosis, reactive oxygen species
Introduction

Neuropathic pain refers to the douleur anormale (abnormal pain), caused by damage and dysfunction of the central or peripheral nervous system[1, 2]. Neuropathic pain is one type of chronic pain, whose main clinical manifestations include hyperalgesia, paresthesia, ambulatory pain and allodynia[3, 4]. According to statistics, the prevalence rate is 0.6-1.5% and with the aging of the population, the prevalence rate increases gradually[5]. At present, the treatment of neuropathic pain is mainly through drug therapy, while there are a number of side effects, and the curative effect is low[6]. Currently, only 30 to 40% of patients have greater than 50% pain relief from medication[7]. This shows that the therapeutic modes of neuropathic pain are not ideal in clinical practice. Therefore, exploring the pathogenesis of neuropathic pain might provide reliable basis and important medical insights into the treatment of pain. There is a growing body of studies indicating that apoptosis of dorsal root ganglion (DRG) neurons is involved in the development of neuropathic pain[8], but its mechanism is still unclear.

In recent years, studies have revealed that reactive oxygen species (ROS) plays a crucial role in the development of neuropathic pain[9, 10]. ROS scavengers, such as phenyl-tert-butyl nitrone (PBN), vitamin E and edaravone can alleviate the manifestations of neuropathic pain in animal models[11, 12]. Moreover, it has been shown that intrathecal injection of ROS scavenger has the most significant effect[13]. It has been proved that intracellular ROS can activate many signaling pathways and induce cell death[14, 15]. Therefore, induction of DRG neurons apoptosis may be a vital mechanism for ROS to participate in neuropathic pain[16]. Anaerobic glycolysis is a way for cells to provide energy in response to stressors, such as inflammation and hypoxia[17]. Studies have also demonstrated that in the process of anaerobic glycolysis, a large quantity of ROS could be produced in the cells, and the accumulation of ROS leads to the induction of pain and with advancing age it
could lead to oxidative stress and even death[18, 19]. However, the specific mechanism of anaerobic glycolysis on neuropathic pain hasn’t been fully elucidated.

Dexmedetomidine is a new type of high selectivity α2-adrenergic agonist, with the characteristics of sedation, analgesia, anti-anxiety, inhibition of sympathetic activity, maintenance of hemodynamic stability and no respiratory inhibition[20]. Currently, dexmedetomidine has been used as a safe and effective drug in clinical practice[21]. In 2009, the US Food and Drug Administration has approved dexmedetomidine to be used for sedation during tracheal intubation and mechanical ventilation in patients undergoing general anesthesia. At present, because of its unique properties, it can be administered before anesthesia, or as a general or local anesthesia adjuvant, and it can also be used in the perioperative period of sedation and analgesia[20]. At present, studies have examined the effects of dexmedetomidine on antioxidant stress, amino acid toxicity and the inhibition ROS synthesis[22-24]. However, there are no studies showing the regulatory effect of dexmedetomidine on anaerobic glycolysis in cells, and whether it has an alleviating effect on pain in DRG neurons.

In this study, we successfully isolated rat DRG neurons and we explored the influence of dexmedetomidine on H2O2-induced apoptosis, ROS and anaerobic glycolysis. In addition, we further verified the effects of ROS inhibitor (NAC) on H2O2-induced apoptosis and anaerobic glycolysis in rat DRG neurons.

Material and Methods

Animals

A total of 10 Wistar rats (3-week-old) were purchased from the Medical Experimental Animal Center of Guangzhou University of Traditional Chinese Medicine. Before the experiments, the purchased rats were kept in a suitable environment for seven days. In the laboratory, the rats had plenty of food and water, the temperature
was kept at 20-25°C, the humidity was kept at 50-60%, and the light was maintained for 12 hrs every day. This study was performed with the approval of the Institutional Animal Care and Use Committee of Sun Yat-sen University, China, and in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals.

**Cell culture**

DRG neurons were separated from the Wistar rats. The rats were decapitated after being anesthetized with isoflurane. The spine was removed from the dorsal side and DRGs were collected from the spinal cord and placed in cold F12 medium (Biochrom, Germany). Next, the spinal cord was cut and the capsule was opened under the microscope and the capsules were incubated in F12 medium (0.9 mL) and collagenase (0.1 mL, 2612.5 U/mL) for 45 mins at 37°C with 5% CO2. After washing, DRGs were trypsinized and cultured in F12 medium and 10% fetal calf serum (FBS) in an incubator. After 3 days, the cell morphology was observed under an inverted microscope.

**Cell treatment**

Rat DRG neurons were treated with 0, 100, 200, 300, 400 and 500 μM dexmedetomidine for 24 hrs. Following treatment with 100 μM H2O2 for 4 hrs[25], rat DRG neurons were treated with dexmedetomidine (1/10 IC50) or 5 mM N-Acetyl-L-cysteine (NAC; Amresco), respectively.

**CCK-8 assay**

Cell viability was measured using CCK-8 solution (Dojindo Biochemicals, Kumamoto, Japan). The treated rat DRG neurons (5000/well) were seeded in 96-well culture plates. After incubation for 24 hrs, 10 μl CCK-8 solution was added to each well. After 2 hrs, a microplate reader (Bio-Rad, MA, USA) was used to assess the
light absorbance. Following this, the 50% inhibitory concentration (IC50) was calculated. Based on the IC50, we adopted 1/10 IC50 for subsequent experiments.

**Western blotting analysis**

RIPA buffer (Beyotime, Shanghai, China) was utilized to extract proteins from the treated DRG neurons. BCA Protein Assay Kit (Thermo, Cat no. 233225) was applied to examine the concentration of protein in each group. The extracted proteins (30 μg) were separated by 10% SDS-PAGE and transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore). The membranes were then blocked with 5% non-fat dry milk and incubated with primary antibodies at 4 °C overnight. After washing, the membranes were incubated with the secondary antibody (1/3000, Abcam, ab205719). The blots were obtained by using the enhanced chemiluminescence (Thermo Scientific). The primary antibodies contain anti-GAPDH (1/2000, Santa Cruz), anti-NADPHase (1/1000, Abcam, ab27942), anti-HK2 (1/1000, Abcam, ab104836), anti-PFK1 (1/1000, Abcam, ab170868), anti-PK2, (1/1000, Abcam, ab76747), anti-Glut1 (1/1000, Abcam, ab40084), anti-LDHA, (1/1000, Abcam, ab125683), anti-PDK1 (1/1000, Abcam, ab110025), anti-Bax, (1/1000, Abcam, ab53154) and anti-Bcl-2 (1/1000, Abcam, ab196495), antibodies.

**Immunofluorescence (IF) assay**

The coverslips were placed into 6-well plates, and the treated DRG neurons were seeded on the coverslips. The cells were cultured at 37 °C with 5% CO2 for 6 hrs. After washing, the coverslips were fixed with 4% paraformaldehyde (Sigma, Cat# P6148) for 30 mins and washed with PBS for 3 times. Then cells were ruptured
using 50 μl 0.2% Triton X-100 and washed with PBS for 3 times. The cells were blocked by 1 ml 1% BSA for 1 h and then treated with primary antibody MAP2 (Abcam, ab32454) for 2 hrs at room temperature and Goat Anti-Mouse IgG H&L (Alexa Fluor® 647, Abcam, ab150115) for 1.5 hrs at room temperature. After washing, the coverslips and incubated with DAPI (Sigma, cat# NY-S347T) for 10 mins. The stained cells were observed under a laser confocal microscope and the images were collected.

**Cell apoptosis assay**

Cell apoptosis was evaluated using Annexin V-FITC/PI Apoptosis Kit (Abnova, cat# KA3805). The treated DRG neurons were collected in a 10 ml centrifuge tube and centrifuged (1000×g for 5 mins). After washing with PBS, the treated DRG neurons were recollected. The cells were incubated with 5 μL of Annexin V-FITC for 10 mins at room temperature in dark. Then the cells were incubated in 5 μL PI solution at room temperature in dark. The apoptotic cells were assessed using a FACSCalibur Flow Cytometer (Becton Dickinson, San Jose, CA, USA).

**Flow cytometry for ROS expression**

According to previous research[26], the fluorescent dye DHE was used to examine the ROS level. The DRG neurons (1 × 10^6 cells) were treated with 2.5 mmol/L DHE for 25 mins at 37 °C. After washing with PBS, cells were collected and stained with red fluorescence dye. Finally, the results were obtained using flow cytometry.

**Glucose measure**
Glucose was examined by Glucose Uptake Colorimetric Assay Kit (Elabscience, cat#E-BC-K268). Glucose standards were prepared according to experimental instructions. A total of 8 different concentration standards and samples were added to the 96-well plate. The 300 μL working enzyme solution was added to each well, and the 96-well plate was incubated for 15 mins at 37 °C. The OD values were obtained using a microplate reader at 505 nm. The level of glucose was calculated according to the OD values.

**Pyruvic acid detection**

The level of pyruvic acid was confirmed by Pyruvate Assay Kit (Nanjing Jiangcheng Bioengineering Institute, Nanjing, China; cat#A081). Briefly, according to the experimental instructions, the reagents were mixed and incubated for 5 mins. The OD values were assessed using a microplate reader at 505 nm and the level of pyruvic acid was analyzed.

**Lactic acid detection**

The level of lactic acid was determined by lactic Acid assay kit (Nanjing Jiangcheng Bioengineering Institute, Nanjing, China; cat#A019-2). Similarly, following the instructions, all reagents were mixed and incubated for 10 mins at 37 °C. The OD values were evaluated using a microplate reader at 530 nm. The level of lactic acid was calculated based on the OD values.

**ATP/ADP detection**
ATP/ADP ratio was measured by ADP/ATP Ratio Assay Kit (Abnova, cat# KA1673). The treated DRG neurons (1 × 10⁴ cells) were cultured in a microwell plate. ATP reagent was prepared at the following concentration: 95μL assay buffer, 1μL substrate, 1μL co-substrate and 1μL ATP enzyme. Added 90 μL ATP reagent in each well and incubated for 1 min and the Relative Light Units (RLU A) were obtained. ADP reagent was prepared at the following dilution: 5 μL double steamed water and 1μL ADP Enzyme and the RLU B were obtained. ATP/ADP= (RLU A)/ ((RLU C) - (RLU B)).

Statistical analysis

All experiments were repeated three times, the results were displayed as mean ± standard deviation (SD). and the statistical analysis was performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA) with one-way analysis of variance (ANOVA).

Results

Identification of rat DRG neurons

To study neuropathic pain, we isolated rat DRG neurons. The cellular morphology of DRG neurons was as follows: cells demonstrated a round morphology with large somas and several protuberances and there were also a small number of glial cells and non-neuronal nuclei (Figure 1A). In addition, we used IF assay to examine MAP2 expression in rat DRG neurons, and the results showed that the positive expression rate of MAP2 was more than 80% in rat DRG neurons, suggesting that the effect of cell isolation was good (Figure 1B).
Concentration screening of dexmedetomidine

To explore the therapeutic effect of dexmedetomidine on neuropathic pain, rat DRG neurons were treated with different concentrations of dexmedetomidine for 24 hrs and cell proliferation was calculated. The results revealed that dexmedetomidine could inhibit DRG neuron proliferation, and the IC50 of dexmedetomidine was 208.4 μM (Figure 1C). In our subsequent experiments, we treated the rat DRG neurons with 1/10 IC50 (20.84 μM) dexmedetomidine.

Dexmedetomidine inhibited H2O2-induced apoptosis and ROS production in rat DRG neurons

Following treatment with H2O2, rat DRG neurons were treated with dexmedetomidine (1/10 IC50, 20.84 μM). Annexin V FITC/PI staining demonstrated that cell apoptosis was dramatically enhanced in the H2O2-treated group compared with the control group. However, cell apoptosis was markedly reduced in the H2O2+dexmedetomidine group relative to the H2O2 group (P < 0.05, P < 0.01, Figure 2A and 2C). Meanwhile, the ROS level was higher in the H2O2 group compared to the control group; whereas the ROS level was lower in H2O2+dexmedetomidine group compared to the H2O2 group (P < 0.05, P < 0.01, Figure 2B and 2C). We also found that H2O2 upregulated Bax expression and downregulated Bcl-2 expression, while dexmedetomidine could reverse Bax and Bcl-2 expressions mediated by H2O2 in rat DRG neurons (Figure 2D).

Dexmedetomidine suppressed H2O2-induced anaerobic glycolysis in rat DRG neurons
To further analyze the possible mechanism of action of dexmedetomidine on neuropathic pain, we investigated the changes of anaerobic glycolysis in rat DRG neurons, induced by H2O2. The results from Western blot assay revealed that NADPHase, HK2, PFK1, PK2, Glut1, LDHA and PDK1 expressions were markedly increased in the H2O2 group compared to the control group, whereas NADPHase, HK2, PFK1, PK2, Glut1, LDHA and PDK1 expressions were markedly decreased in the H2O2+dexmedetomidine group when compared to the H2O2 group (Figure 3A). In addition, glucose consumption, pyruvic acid and lactic acid expression levels were analyzed, and the results indicated that glucose consumption, pyruvic acid and lactic acid levels were significantly increased in the H2O2 group compared to the control group, while they were significantly reduced in the H2O2+dexmedetomidine group in relation to the H2O2 group (P< 0.05, P< 0.01, Figure 3B-3D). Simultaneously, we also found that the ATP/ADP ratio was significantly decreased in H2O2 group compared to the control group, while it was significantly increased in H2O2+dexmedetomidine group compared to the H2O2 group (P< 0.05, P< 0.01, Figure 3E). Therefore, we determined that dexmedetomidine could inhibit H2O2-mediated anaerobic glycolysis in rat DRG neurons.

**Dexmedetomidine reduced H2O2-induced apoptosis by inhibiting ROS in rat DRG neurons**

ROS participates in many cellular metabolic processes, such as growth, proliferation and apoptosis by regulating various signaling pathways[27, 28]. To further study the inhibitory effects of dexmedetomidine on H2O2-induced apoptosis, we adopted ROS inhibitor (N-Acetyl-L-cysteine, NAC) to treat the H2O2-induced rat DRG neurons. The results of Annexin V FITC/PI staining demonstrated that both dexmedetomidine and NAC significantly inhibited H2O2-induced cell apoptosis in rat DRG neurons (P< 0.05, P< 0.01, Figure 4A and 4C). We also demonstrated that both dexmedetomidine and NAC significantly reduced H2O2-induced ROS levels in rat DRG neurons (P< 0.05, P< 0.01, Figure 4B and 4C). In addition, we found that, when compared...
with H₂O₂ group, both dexmedetomidine and NAC dramatically decreased Bax expression, and increased Bel-2 expression (Figure 4D). Therefore, we determined that dexmedetomidinemight be involved in the suppression of ROS in H₂O₂-induced apoptosis in rat DRG neurons.

**Dexmedetomidine attenuated anaerobic glycolysis by inhibiting ROS in rat DRG neurons**

Furthermore, we verified the influence and mechanism of dexmedetomidine on H₂O₂-mediated anaerobic glycolysis in rat DRG neurons. As shown in Figure 5A, both dexmedetomidine and NAC downregulated the protein expression levels of NADPHase, HK2, PFK1, PK2, Glut1, LDHA and PDK1, which they were induced by H₂O₂ in rat DRG neurons. At the same time, we determined that both dexmedetomidine and NAC could inhibit the levels of glucose consumption, pyruvic acid, lactic acid, which were found to be induced by H₂O₂.

We also found that dexmedetomidine enhanced the ATP/ADP ratio, which was smothered by H₂O₂, in rat DRG neurons (P< 0.05, P< 0.01, Figure 5B-5E). So, we concluded that the inhibition of anaerobic glycolysis by dexmedetomidine was closely associated with ROS inhibition, in rat DRG neurons.

**Discussion**

Peripheral nerve injury often leads to refractory neuropathic pain[2, 29]. Studies using animal model have demonstrated that neuropathic pain induced by peripheral nerve injury is often accompanied by apoptosis of spinal DRG neurons[30, 31]. A large number of studies have also confirmed that apoptosis of spinal DRG neurons was involved in the process of neuropathic pain[32, 33], but the mechanism is still unclear. In our study, we successfully isolated and identified rat DRG neurons, as shown in previous studies [34].
ROS are free radicals produced by organisms, which include active singlet oxygen, hydrogen peroxide and other oxygen free radicals[35]. Once these free radicals are produced, they can be transformed into each other, which will cause oxidative damage to macromolecules, hyperoxidative degeneration, cross-linking or fracture, destruction of cellular structure and function, leading to tissue damage. These injuries have been shown to be the basis of aging, neurodegenerative diseases, and tumorigenesis[36]. At present, the removal of excessive ROS has also been considered as an effective treatment for neuropathic pain[37]. The mechanisms of pain relief mainly includes increased intracellular Ca2+, phosphorylation of NMDA receptors and up-regulation of apoptotic genes, such as bax, caspase-3, caspase-9 and apoptotic protease-activating factor-1 (Apaf-1). In recent years, several studies have suggested that ROS was closely related to a variety of neurodegenerative diseases including amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease, and injury or aging-related brain dysfunction[38, 39]. In addition, a number of studies have revealed that ROS was involved in the apoptosis related to these conditions [40, 41]. Other researchers have also demonstrated that low concentrations of H2O2 can induce apoptosis in a variety of cells[42, 43]. In addition, research has also found that dexmedetomidine exhibits an anti-oxidative stress effect by significantly inhibiting the excessive production of ROS. In our study, we further demonstrated that in rat DRG neurons, dexmedetomidine could inhibit H2O2-induced apoptosis and reduce the level of ROS.

Glycolysis is a process in which glucose or glycogen is degraded to eventually form lactic acid or pyruvate in tissues, while releasing some energy in the form of ATP to be utilized by the tissues [44]. Anaerobic glycolysis refers to the process by which glucose or glycogen decomposes into lactic acid and generates energy, when the body is in a relatively anoxic condition[17]. Therefore, the main indicators of anaerobic glycolysis include glucose consumption, lactic acid and pyruvic acid production and ATP/ADP ratio. These are the markers used in studies of cell anaerobic glycolysis [45, 46]. In our study, we found that dexmedetomidine significantly
reduced H$_2$O$_2$-induced glucose consumption, pyruvic acid and lactic acid expression as well as increased the ATP/ADP ratio, in rat DRG neurons. As for the mechanism of action, previous studies have suggested that cells can transport sugars from the extracellular region to the intracellular by glucose transporter (GLUT), which is very important for glucose metabolism[47]. Lactate dehydrogenase A (LDHA) can convert pyruvate into lactic acid, which provides ATP for the body[48]. Pyruvate dehydrogenase 1 (PDK1) is an essential enzyme for pyruvate dehydrogenation by the mitochondria[49]. In addition, several enzymes involved in glycolysis, were found to be elevated, such as pyruvate kinase 2 (PK2), phosphofructokinase 1 (PFK1), hexokinase 2 (HK2), in disease[50]. In our study, we also demonstrated that dexmedetomidine markedly decreased NADPHase, HK2, PFK1, PK2, Glut1, LDHA and PDK1 expression, which were induced by H$_2$O$_2$, in rat DRG neurons. Therefore, we have shown that dexmedetomidine could dramatically suppress H$_2$O$_2$-induced anaerobic glycolysis, in rat DRG neurons.

A previous study has indicated that under stress, anaerobic glycolysis of cells can lead to an increase in the production of ROS, and the accumulated ROS can further induce pain[51]. Another study has also demonstrated that inhibition of anaerobic glycolysis could reduce systolic function and mechanical efficiency, while promotion of glycolysis and glucose oxidation could improve the systolic function during ischemia by inhibiting the oxidation of free fatty acids[52]. In our study, we found that treatment with either dexmedetomidine or a ROS inhibitor (NAC) could reduce H$_2$O$_2$-induced ROS synthesis and apoptosis as well as the levels of glucose consumption, pyruvic acid and lactic acid, in H$_2$O$_2$-induced rat DRG neurons. In combination with the inhibitory effect of dexmedetomidine on ROS in H$_2$O$_2$-induced rat DRG neurons, we further demonstrated that dexmedetomidine could reduce H$_2$O$_2$-induced apoptosis and anaerobic glycolysis by inhibiting ROS in rat DRG neurons.
In conclusion, our study has demonstrated that dexmedetomidine inhibited H$_2$O$_2$-induced apoptosis, ROS production and anaerobic glycolysis, similar to a ROS inhibitor (NAC), in rat DRG neurons.
References

1. Zilliox, L.A., *Neuropathic Pain*. Continuum (Minneap Minn), 2017. 23(2, Selected Topics in Outpatient Neurology): p. 512-532.

2. Hu, Y., et al., *MicroRNA-362-3p attenuates motor deficit following spinal cord injury via targeting paired box gene 2*. Journal of integrative neuroscience, 2019. 18(1): p. 57-64.

3. William, R., et al., *Population-based study of central post-stroke pain in Rimini district, Italy*. 2013.

4. Watson, J.C. and P. Sandroni, *Central Neuropathic Pain Syndromes*. Mayo Clin Proc, 2016. 91(3): p. 372-85.

5. Ahmed, S.U., et al., *Effect of 1.5% Topical Diclofenac on Clinical Neuropathic Pain*. 2016. 60(1): p. 191.

6. Xu, L., Y. Zhang, and Y. Huang, *Advances in the Treatment of Neuropathic Pain*. Adv Exp Med Biol, 2016. 904: p. 117-29.

7. Laufenberg-Feldmann, R., et al., *Anxiety and its predictive value for pain and regular analgesic intake after lumbar disc surgery - a prospective observational longitudinal study*. 2018. 18(1): p. 82.

8. Widerstrom-Noga, E., *Neuropathic Pain and Spinal Cord Injury: Phenotypes and Pharmacological Management*. Drugs, 2017. 77(9): p. 967-984.

9. Bittar, A., et al., *Reactive oxygen species affect spinal cell type-specific synaptic plasticity in a model of neuropathic pain*. Pain, 2017. 158(11): p. 2137-2146.

10. Hassler, S.N., K.M. Johnson, and C.E. Hulsebosch, *Reactive oxygen species and lipid peroxidation inhibitors reduce mechanical sensitivity in a chronic neuropathic pain model of spinal cord injury in rats*. J Neurochem, 2014. 131(4): p. 413-7.
11. Kim, H.K., et al., *Phenyl N-tert-butylnitrone, a free radical scavenger, reduces mechanical allodynia in chemotherapy-induced neuropathic pain in rats.* 2010. **112**(2): p. 432.

12. Mao, Y.F., et al., *Edaravone, a free radical scavenger, is effective on neuropathic pain in rats.* 2009. **1248**(none): p. 68-75.

13. Kim, H.Y., et al., *Reactive Oxygen Species Donors Increase the Responsiveness of Dorsal Horn Neurons and Induce Mechanical Hyperalgesia in Rats.* Neural Plast, 2015. **2015**: p. 293423.

14. Holze, C., et al., *Oxeiptosis, a ROS-induced caspase-independent apoptosis-like cell-death pathway.* Nat Immunol, 2018. **19**(2): p. 130-140.

15. Zhao, Y., et al., *ROS signaling under metabolic stress: cross-talk between AMPK and AKT pathway.* Mol Cancer, 2017. **16**(1): p. 79.

16. Ding, R., et al., *Advanced Oxidative Protein Products Cause Pain Hypersensitivity in Rats by Inducing Dorsal Root Ganglion Neurons Apoptosis via NADPH Oxidase 4/c-Jun N-terminal Kinase Pathways.* Front Mol Neurosci, 2017. **10**: p. 195.

17. Peek, C.B., et al., *Circadian Clock Interaction with HIF1alpha Mediates Oxygenic Metabolism and Anaerobic Glycolysis in Skeletal Muscle.* Cell Metab, 2017. **25**(1): p. 86-92.

18. Jian, S.L., et al., *Glycolysis regulates the expansion of myeloid-derived suppressor cells in tumor-bearing hosts through prevention of ROS-mediated apoptosis.* Cell Death Dis, 2017. **8**(5): p. e2779.

19. Qin, W., et al., *Inhibition of autophagy promotes metastasis and glycolysis by inducing ROS in gastric cancer cells.* Oncotarget, 2015. **6**(37): p. 39839-54.

20. Devasya, A. and M. Sarpangala, *Dexmedetomidine: A Review of a Newer Sedative in Dentistry.* J Clin Pediatr Dent, 2015. **39**(5): p. 401-9.
21. Keating, G.M., *Dexmedetomidine: A Review of Its Use for Sedation in the Intensive Care Setting*. Drugs, 2015. **75**(10): p. 1119-30.

22. Chen, Y., et al., *Dexmedetomidine Ameliorates Acute Stress-Induced Kidney Injury by Attenuating Oxidative Stress and Apoptosis through Inhibition of the ROS/JNK Signaling Pathway*. Oxid Med Cell Longev, 2018. **2018**: p. 4035310.

23. Cui, J., et al., *Dexmedetomidine attenuates oxidative stress induced lung alveolar epithelial cell apoptosis in vitro*. Oxid Med Cell Longev, 2015. **2015**: p. 358396.

24. Huang, J. and Q. Jiang, *Dexmedetomidine Protects Against Neurological Dysfunction in a Mouse Intracerebral Hemorrhage Model by Inhibiting Mitochondrial Dysfunction-Derived Oxidative Stress*. J Stroke Cerebrovasc Dis, 2019. **28**(5): p. 1281-1289.

25. Mohiuddin, M.S., et al., *Glucagon-Like Peptide-1 Receptor Agonist Protects Dorsal Root Ganglion Neurons against Oxidative Insult*. Journal of diabetes research, 2019. **2019**.

26. Shen, Y., et al., *Protective effects of hydrogen sulfide in hypoxic human umbilical vein endothelial cells: a possible mitochondria-dependent pathway*. Int J Mol Sci, 2013. **14**(7): p. 13093-108.

27. Behrend, L., G. Henderson, and R. Zwacka, *Reactive oxygen species in oncogenic transformation*. 2003, Portland Press Ltd.

28. Diwanji, N. and A. Bergmann, *The beneficial role of extracellular reactive oxygen species in apoptosis-induced compensatory proliferation*. Fly, 2017. **11**(1): p. 46-52.

29. Dewandre, Q., et al., *Refractory neuropathic pain from a median nerve injury: spinal cord or peripheral nerve stimulation? A case report*. 2019: p. 1-5.

30. Lim, H., et al., *IKK/NF-κB-dependent satellite glia activation induces spinal cord microglia activation and neuropathic pain after nerve injury*. 2017. **158**(9): p. 1666-1677.
31. Liu, C.N., et al., Spinal Nerve Injury Enhances Subthreshold Membrane Potential Oscillations in DRG Neurons: Relation to Neuropathic Pain. 2000. 84(1): p. 205.

32. Ding, R., et al., Advanced Oxidative Protein Products Cause Pain Hypersensitivity in Rats by Inducing Dorsal Root Ganglion Neurons Apoptosis via NADPH Oxidase 4/c-Jun N-terminal Kinase Pathways. 2017. 10: p. 195-.

33. Özdemir, Ü.S., et al., Hypericum perforatum Attenuates Spinal Cord Injury-Induced Oxidative Stress and Apoptosis in the Dorsal Root Ganglion of Rats: Involvement of TRPM2 and TRPV1 Channels. 2015. 53(6): p. 1-12.

34. Kawai, H., et al., Neurotropin inhibits neuronal activity through potentiation of sustained Kv currents in primary cultured DRG neurons. 2018: p. S1347861318300860-.

35. Griesbeck, A.G., B. Öngel, and M.J.J.o.P.O.C. Atar, New phthalimide-methionine dyad-based fluorescence probes for reactive oxygen species: Singlet oxygen, hydrogen peroxide, and hypochlorite. 2017. 30(9): p. e3741.

36. Umeno, A., V. Biju, and Y. Yoshida, In vivo ROS production and use of oxidative stress-derived biomarkers to detect the onset of diseases such as Alzheimer’s disease, Parkinson’s disease, and diabetes. Free Radic Res, 2017. 51(4): p. 413-427.

37. Magar, S., et al., Ultra-diluted Toxicodendron pubescens attenuates pro-inflammatory cytokines and ROS-mediated neuropathic pain in rats. Sci Rep, 2018. 8(1): p. 13562.

38. Deshmukh, P., et al., The Keap1-Nrf2 pathway: promising therapeutic target to counteract ROS-mediated damage in cancers and neurodegenerative diseases. Biophys Rev, 2017. 9(1): p. 41-56.

39. Liu, Z., et al., Oxidative Stress in Neurodegenerative Diseases: From Molecular Mechanisms to Clinical Applications. Oxid Med Cell Longev, 2017. 2017: p. 2525967.
40. Diwanji, N. and A. Bergmann, *An unexpected friend - ROS in apoptosis-induced compensatory proliferation: Implications for regeneration and cancer*. Semin Cell Dev Biol, 2018. **80**: p. 74-82.

41. Zhang, M., et al., *The Roles of ROS and Caspases in TRAIL-Induced Apoptosis and Necroptosis in Human Pancreatic Cancer Cells*. PLoS One, 2015. **10**(5): p. e0127386.

42. Gutierrez-Venegas, G., et al., *Hydrogen peroxide-induced apoptosis in human gingival fibroblasts*. Int J Clin Exp Pathol, 2015. **8**(12): p. 15563-72.

43. Hu, J., et al., *Globular Adiponectin Attenuated H2O2-Induced Apoptosis in Rat Chondrocytes by Inducing Autophagy Through the AMPK/ mTOR Pathway*. Cell Physiol Biochem, 2017. **43**(1): p. 367-382.

44. Donnelly, R.P. and D.K. Finlay, *Glucose, glycolysis and lymphocyte responses*. Mol Immunol, 2015. **68**(2 Pt C): p. 513-9.

45. Wu, K.C., et al., *Perturbation of Akt Signaling, Mitochondrial Potential, and ADP/ATP Ratio in Acidosis-Challenged Rat Cortical Astrocytes*. 2017. **118**(5).

46. Duan, K., et al., *Lactic acid induces lactate transport and glycolysis/OXPHOS interconversion in glioblastoma*. 2018. **503**(2): p. S006291X18314049-.

47. Szablewski, L., *Glucose Transporters in Brain: In Health and in Alzheimer’s Disease*. J Alzheimers Dis, 2017. **55**(4): p. 1307-1320.

48. Mack, N., et al., *Stable shRNA Silencing of Lactate Dehydrogenase A (LDHA) in Human MDA-MB-231 Breast Cancer Cells Fails to Alter Lactic Acid Production, Glycolytic Activity, ATP or Survival*. Anticancer Res, 2017. **37**(3): p. 1205-1212.

49. Tan, Z., et al., *Pyruvate dehydrogenase kinase 1 participates in macrophage polarization via regulating glucose metabolism*. J Immunol, 2015. **194**(12): p. 6082-9.
50. Wang, Y., et al., *Qiliqiangxin attenuates hypoxia-induced injury in primary rat cardiac microvascular endothelial cells via promoting HIF-1α-dependent glycolysis*. 2018.

51. Wakiyama, K., et al., *Low-dose YC-1 combined with glucose and insulin selectively induces apoptosis in hypoxic gastric carcinoma cells by inhibiting anaerobic glycolysis*. Sci Rep, 2017. 7(1): p. 12653.

52. Zhou, L., et al., *Impact of anaerobic glycolysis and oxidative substrate selection on contractile function and mechanical efficiency during moderate severity ischemia*. Am J Physiol Heart Circ Physiol, 2008. 295(3): p. H939-h945.
Figure legends

Figure 1. Identification of rat DRG neurons and concentration screening of dexmedetomidine.

(A) Cultured rat DRG neurons were observed using an inverted microscope, magnification, 100×. (B) MAP2 expression was elicited by IF assay, in rat DRG neurons, magnification, 100×, scale bar = 100 μm. (C) Rat DRG neurons were treated with 0, 100, 200, 300, 400 and 500 μM dexmedetomidine for 24 hrs. Cell proliferation was determined by CCK-8 assay, and IC50 was calculated.

Figure 2. Dexmedetomidine inhibited apoptosis and ROS production in H2O2 induced rat DRG neurons.

Rat DRG neurons were treated with dexmedetomidine and H2O2. (A) Annexin V FITC/PI staining was used to determine cell apoptosis. (B) The level of ROS was analyzed using a Flow cytometer with DCFH-DA fluorescent probe. (C) The apoptosis rate and ROS levels were quantitatively calculated (**P < 0.01 vs. normal group; #P < 0.05 vs. Dex group). (D) Bax and Bcl-2 expressions were determined by western blot analysis, GAPDH acted as an internal control.

Figure 3. Dexmedetomidine suppressed anaerobic glycolysis in H2O2 induced rat DRG neurons.

Dexmedetomidine and H2O2 were used to treat rat DRG neurons. (A) Western blot assay was performed to determine the levels of NADPHase, HK2, PFK1, PK2, Glut1, LDHA and PDK1. GAPDH served as a control for normalization. (B) Glucose consumption was analyzed using Glucose Uptake Colorimetric Assay Kit. (C) The level of pyruvic acid was measured using Pyruvate Assay Kit. (D) The concentration of lactic acid was determined by lactic Acid assay kit. (E) The ATP/ADP ratio was confirmed by ADP/ATP Ratio Assay Kit. **P < 0.01 vs. normal group; #P < 0.05 vs. H2O2 group.
Figure 4. Dexmedetomidine reduced H$_2$O$_2$-induced apoptosis by inhibiting ROS in rat DRG neurons. Rat DRG neurons were treated with H$_2$O$_2$, dexmedetomidine or/and ROS inhibitor (NAC). (A) Cell apoptosis was examined by Annexin V FITC/PI staining. (B) Flow cytometer with DCFH-DA fluorescent probe was used to evaluate ROS levels. (C) Quantitative analyses of the apoptosis rate and ROS level, **$P<0.01$ vs. normal group; $P<0.05$ vs. H$_2$O$_2$ group. (D) The western blotting analyses of Bax and Bcl-2 expressions.

Figure 5. Dexmedetomidine attenuated anaerobic glycolysis by inhibiting ROS in rat DRG neurons. Rat DRG neurons were treated with H$_2$O$_2$, dexmedetomidine and/or NAC. (A) Western blotting analysis was utilized to assess the protein levels of NADPHase, HK2, PFK1, PK2, Glut1, LDHA and PDK1 in the treated rat DRG neurons. (B-D) Glucose consumption, pyruvic acid and lactic acid expression and ATP/ADP ratios were measured using the corresponding kits, **$P<0.01$ vs. normal group; $P<0.05$ vs. H$_2$O$_2$ group.
A Normal
Dex
H$_2$O$_2$
H$_2$O$_2$+Dex

B Normal
Dex
H$_2$O$_2$
H$_2$O$_2$+Dex

C

D

Bax
Bcl-2
GAPDH
