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A Comparative Study on the Antinociceptive Effects of Phosphodiesterase Inhibitors on Sciatic Nerve Ligation Induced Neuropathic Pain in Mice

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Abstract

Introduction: The role of phosphodiesterase (PDE) inhibitors in reducing neuropathic pains is uncertain. In this study, the antinociceptive effects of theophylline, milrinone, and tadalafil were investigated on sciatic nerve ligation-induced neuropathic pain (NP).

Methods: Male mice (25-30 g) were purchased and housed in controlled environmental conditions before and during the experiments. The mice received identical diet and water *ad libitum*. Two weeks after sciatic nerve ligation, either theophylline (75 mg/kg), milrinone (4.5 mg/kg), or tadalafil (20 mg/kg) was intraperitoneally (IP) injected for either 1, 3, or 7 consecutive days. Antinociceptive effects were evaluated using the hot plate test. Negative controls received time course IP injections of saline (5 mL/kg). A single dose of imipramine (40 mg/kg,) was intraperitoneally administrated to the mice in the positive control group.

Results: As was found for this study, a single-dose IP injection of either theophylline (75 mg/kg), milrinone (4.5 mg/kg), or tadalafil (20 mg/kg) on day 14th following sciatic nerve ligation induced significant antinociceptive effects at 30 minutes ($P<0.01$), 60 minutes ($P<0.01$), and 90 minutes ($P<0.05$) compared to the control (saline– treated) animals. Accordingly, both 3- (on days 12–14) and 7-day (on days 8– 14) IP injections of tadalafil (20 mg/kg) induced significant antinociceptive effects at 30 minutes ($P<0.05$), 60 minutes ($P<0.01$), and 90 minutes ($P<0.01$) after sciatic nerve ligation compared to the control (saline–treated) animals. However, the 3- and 7-day IP injections of theophylline and milrinone did not reveal any significant differences compared to the control group.

Conclusion: Taken together, the results of this study suggested that selective PDE inhibitors that act predominantly on cGMP pathway, may contribute to the management of sciatic nerve ligation–induced pain.

Keywords: Neuropathic pain, Hot plate test, Phosphodiesterase inhibitors, Imipramine, Mice

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Introduction

Neuropathic pain (NP) as a primary lesion or dysfunction of the nervous system may be a peripheral or central dysfunction. The peripheral nerves, the plexus, dorsal nerve roots, the spinal cord, and brain are the most commonly influenced organs in this model of pain.[1] The etiology and pathophysiological pathways involved in NP induced by somatosensory lesions in the peripheral or central nervous system (CNS) are largely unknown.[1-3] NP is characterized by allodynia, hyperalgesia, and paresthesia.[4] Marked degeneration of both myelinated A-fibers and unmyelinated C-fibers as a result of chronic nerve constriction mimics some aspects of NP in humans.[1] The hot plate test is a behavioral model of nociception which is usually used in monitoring the effects of pain-relieving drugs.[5]

Despite multiple studies on the

neurobiological mechanism of chronic pain, there are few effective therapies for this type of pain. The principal mechanisms are weakly understood and often noncompliant to conventional antinociceptives such as opiates (limited efficacy in this pain model) and non-steroidal anti-inflammatory drugs.[1]

Phosphodiesterase (PDE) enzymes regulate the levels of secondary messengers viz cAMP and cGMP, within cells.[6] Eleven isoenzyme families of PDEs have been recognized with a wide range of functions at cellular and molecular levels. These enzymes promote the substantial effects in the pathogenesis of various diseases, especially through modulating cGMP levels.[7] It has been reported that elevated levels of cGMP are associated with inhibition of tissue hyperalgesia.[6,8,9] Considerable body of evidence has assessed the pivotal role of the balance between cAMP and cGMP in the genesis of tissue hyperalgesia.[10-12]

The antinociceptive effects of milrinone as a PDE-4 inhibitor have been reported previously.[6] Furthermore, it has been reported that theophylline, a nonspecific PDE inhibitor, exerts dose dependent analgesic and anti-inflammatory effects.[13] It has also been demonstrated that tadalafil, as a PDE-5 inhibitor, exhibits antinociceptive and anti-inflammatory activities in animal models of arthritis.[14] The purpose of this study was to evaluate the antinociceptive effects of milrinone, theophylline, and tadalafil on sciatic nerve ligation-induced NP in mice, and to further determine the possible involved biochemical pathways.

Materials and Methods

Animals

This study was carried out in the Department of Pharmacology and Toxicology, Zabol University of Medical Sciences, Zabol, Iran. Male mice (25-30 g) were purchased from the Animal Breeding and Care Center, Department of Pharmacology, Zabol University of Medical Sciences. All the animals were housed in the controlled environmental conditions (25 ± 2°C and 12:12 hours light-dark cycle) before and during the experiments. The mice received identical diet and water *ad libitum*.

Drugs

Theophylline (Darou Pakhsh C., Iran), milrinone (Sanofi-Aventis, France), Tadalafil (OSveh Co., Iran) and imipramine (Sobhan Co., Iran) were dissolved in saline and injected intraperitoneally. Ketamine (Alfasan, Holland) and xylazine (Alfasan, Holland) were used for surgical anesthesia.

Antinociceptive Studies

Hot Plate Test

Pain sensitivity in sciatic nerve ligated mice (a model of NP) was evaluated using the hot plate test. At first, the animals were anesthetized with ketamine (80 mg/kg) and xylazine (20 mg/kg) and their right sciatic nerve was ligated by a copper wire. Afterward, the animals were intraperitoneally injected with theophylline (75 mg/kg), milrinone (4.5 mg/kg), and tadalafil (20 mg/kg). All the drugs, namely, theophylline, milrinone, and tadalafil, were administrated for either 1, 3, or 7 days, respectively. After sciatic nerve ligation (cut-off time was restricted to 45 seconds) within 90 minutes, a duration of 14 days was determined for latency to licking and lifting paws or jumping from the hot plate surface. Negative and positive control animals were IP injected with saline (5 mL/kg) and a single dose of imipramine (40 mg/kg), respectively.

Statistical Analysis

Statistical tests were conducted using SPSS software version 19.0. One-way analysis of variance (ANOVA) followed by Newman–Keuls multiple comparison post–hoc test and independent samples *t* test were used to compare the groups. *P* value <0.05 was considered as statistically significant.

Results

Analgesic Effects of Single-Dose IP injection of PDE Inhibitors to Sciatic Nerve Ligated Mice

As shown in Figure 1 (A-D), a single-dose IP injection of theophylline (75 mg/kg) to the mice on day 14, following sciatic nerve ligation, induced significant antinociceptive effects at 30 minutes (***$P<0.001$), 60 minutes (**$P<0.01$), and 90 minutes (*$P<0.05$) compared to the animals in the control (saline – treated) group. Milrinone

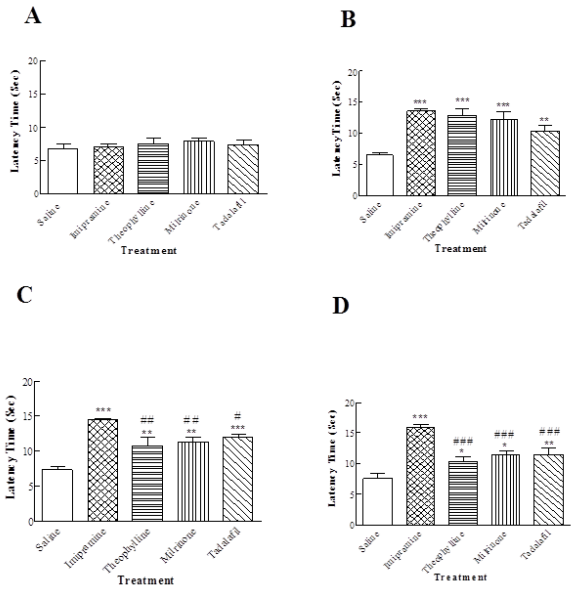

Figure 1. Antinociceptive Effects of Single Dose (Day 14th) IP Injections of PDE Inhibitors, Imipramine (Positive Control), and Saline (Negative Control) to Sciatic Nerve Ligated Mice by Assessing the Latency Response. A, B, C, and D represent assessment of antinociception at 0 (pre-injection), 30, 60, and 90 minutes, respectively, two-weeks after sciatic nerve ligation. Each value represents the mean ± SEM for 7 mice. Note: *$P<0.05$, **$P<0.01$, and ***$P<0.001$ significantly different from the control (saline–treated) animals. #$P<0.05$, ##$P<0.01$, and ###$P<0.001$ significantly different from the imipramine – treated animals.

(4.5 mg/kg) induced significant antinociceptive effects at 30 minutes (***$P<0.001$), 60 minutes (**$P<0.01$), and 90 minutes (*$P<0.05$) after IP injection to the sciatic nerve ligated animals compared to the control (saline–treated) animals. Moreover, in comparison with saline-treated animals (control group), IP injection of tadalafil (20 mg/kg) to the sciatic nerve ligated animals demonstrated significant antinociceptive effects at 30 minutes (**$P<0.01$), 60 minutes (***$P<0.001$), and 90 minutes (**$P<0.01$). In addition, the analgesic effects of imipramine as a positive control were observed at 30 minutes and persisted until 90 minutes after the IP injection (Figure 1 B-D).

Antinociceptive Effects of IP Injections of PDE Inhibitors to Sciatic Nerve Ligated Mice in 3-Day and 7-Day Periods

As shown in Figure 2 (A-D), IP injection of tadalafil (20 mg/kg) for 3 days (on days 12-14) induced significant antinociceptive effects at 30 minutes (***$P<0.001$), 60 minutes (**$P<0.01$), and 90 minutes (**$P<0.01$) after sciatic nerve ligation compared to the control group (saline–treated animals).

Furthermore, as depicted in Figure 3 (A-D), 7-day IP injections of tadalafil (20 mg/kg, on days 8–14) to the mice under this study induced significant antinociceptive effects at 30 minutes (*$P<0.05$), 60 minutes (***$P<0.001$), and 90 minutes (***$P<0.001$) after sciatic nerve ligation compared to the control animals (saline–treated). However, the 3- and 7-day IP injections of theophylline and milrinone did not reveal any significant differences compared to the control animals.

Discussion

The molecular mechanisms of hyperalgesia mediated by the factors such as prostanoids and sympathomimetic amines have been incompletely addressed. There are suggestions indicating a significant role for the balance of Ca^{2+}/cAMP and cGMP neuronal content in this process.[6] In particular, an essential role has been highlighted for cAMP in determining the sensitivity of sensory neurons to hyperalgesic stimulus as evidenced by the action of PDE inhibitors.[6]

The results of the present study indicated that single dose IP injection of theophylline (75 mg/kg, as a non-selective PDE inhibitor), milrinone (4.5 mg/kg, as a PDE4 inhibitor), and tadalafil (20 mg/kg, as a PDE5 inhibitor) induced significant anti-nociceptive effects in the sciatic nerve ligated mice. Findings of this study proved significant differences between all these mentioned PDE inhibitors and imipramine– and saline- treated groups. In addition, both 3- and 7-day IP injections of tadalafil (20 mg/kg) induced significant antinociceptive effects in comparison with the negative control animals (saline–treated). However, 3- and 7-day IP injections of theophylline and milrinone did not cause any significant differences in comparison with the control animals.

Several bodies of evidence have demonstrated that it is possible to modulate hyperalgesia via adjustment of

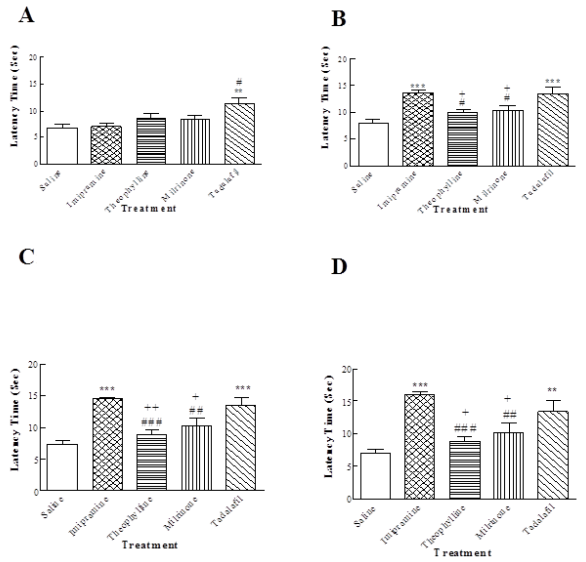

Figure 2. Antinociceptive Effects of 3-Day (days 12th – 14th) IP Injections of PDE Inhibitors, Imipramine (Single Dose), and Saline to Sciatic Nerve Ligated Mice by Assessing the Latency Response. A, B, C, and D represent assessment of antinociception at 0 (pre-injection), 30, 60, and 90 minutes, respectively. Each value represents the mean ± SEM for 7 mice.
Note: **P < 0.01 and ***P < 0.001 significantly different from the control (saline – treated) animals. $^{\#}P$ < 0.05, $^{\#\#}P$ < 0.01, and $^{\#\#\#}P$ < 0.001 significantly different from the imipramine–treated animals. ^{+}P < 0.05 and ^{++}P < 0.01 significantly different from the tadalafil–treated animals.

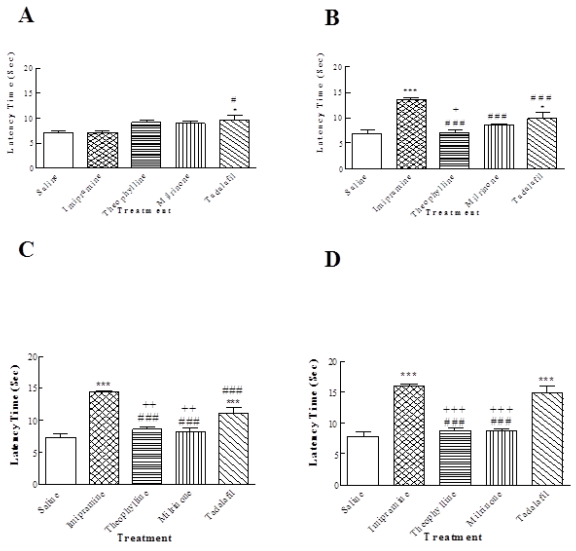

Figure 3. Antinociceptive Effects of 7-Day (Days 8th – 14th) IP Injections of PDE Inhibitors, Imipramine (Single Dose), and Saline to Sciatic Nerve Ligated Mice by Assessing the Latency Response. A, B, C, and D represent assessment of antinociception at 0 (pre-injection), 30, 60, and 90 minutes, respectively. Each value represents the mean ± SEM for 7 mice.
Note: *P < 0.05 and ***P < 0.001 significantly different from the control (saline–treated) animals. $^{\#}P$ < 0.05 and $^{\#\#\#}P$ < 0.001 significantly different from the imipramine – treated animals. ^{+}P < 0.05, ^{++}P < 0.01, and ^{+++}P < 0.001 significantly different from the tadalafil–treated animals.

the intracellular levels of cAMP or cGMP in peripheral sensitive neurons.[8,9,15] Studies have shown that elevated levels of cAMP and cGMP are associated with the increase and decrease in the tissue hyperalgesia, respectively.[6] A considerable body of literature has evaluated the pivotal role of the balance between cAMP and cGMP in the biogenesis of hyperalgesia.[6,10-12] Sildenafil was reported to induce antinociception via the inhibition of cGMP degradation.[8] Furthermore, the contribution of the NO/cGMP signaling pathway has been mentioned in nociception in previous studies.[9]

Conclusion

To conclude, the findings of this study showed a relationship between analgesia in sciatic nerve ligated mice as a model of NP and the activation of cGMP pathway. The balance between the intracellular levels of cAMP and cGMP may stand as a possible candidate for developing new peripheral analgesics.

Ethical Approval

The study protocol was approved by the Local Ethics Committee for Animal Experimentation, Zabol University of Medical Sciences, Zabol, Iran.

Competing Interests

None.

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