Carbenoxolone, a Gap Junction Blocker, Enhances Antinociceptive Effects of Morphine in Formalin Test in Mice

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Summary

In this study, the antinociceptive effects of the gap junction blocker carbenoxolone and morphine were examined using the formalin test as a model of pain. We found that carbenoxolone was dose-dependently antinociceptive in both the first and second phases of the formalin test. Pretreatment with carbenoxolone increased antinociceptive effect of morphine in the first phase of formalin test. Also, pretreatment of carbenoxolone at lower dose of morphine could enhance antinociceptive effect of it in the second phase of formalin test. It is possible that gap junction blockade may be involved in the antinociceptive activity of carbenoxolone.

Keywords: Carbenoxolone, Morphine, Gap junction, Antinociceptive, Formalin test

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Introduction

Carbenoxolone, the succinyl ester of glycyrrhetinic acid [1], is used in the clinical treatment of ulcer diseases [2]. It has some pharmacological properties, such as the inhibition of gap-junctional intercellular communication [3]. Carbenoxolone has anticonvulsant, sedative, and muscle relaxant activity in mice [4].

Intercellular communication via gap junctions is of paramount importance in the regulation of a variety of biological processes. The channels formed by gap junctions allow for intercellular diffusion of small (<1 kDa) hydrophilic molecules and ions [5]. Signaling molecules, including inositol (1,4,5)-triphosphate (IP$_3$), calcium, ATP and cAMP, have been shown to travel via gap junctions in cell lines, and increased intracellular concentrations of cAMP lead to increased gap junction coupling [6-8].

Within the central nervous system, gap junctions play different roles in the brain and spinal cord. In the brain, gap junctions between glial cells have long been thought to contribute to tissue homeostasis [9]. Glial cells also receive information from both primary afferent terminals and pain transmitting neurons, and these cells can be sensitively activated by formalin, injury and a wide array of endogenous substances such as substance P, ATP, excitatory amino acids, nitric oxide and prostaglandins [10-12]. In contrast, in the spinal cord gap junctions are thought to be involved in pain facilitation, suggesting that gap junction activation might lead to proinflammatory cytokine release by distantly activated glia [13]. In our previous study, we have found that quinine, a gap junction blocker, has dose-dependent antinociceptive properties in the first and second phases of the formalin test, although the effect of quinine was greater on the second phase. In contrast, trimethylamine (TMA), a gap junction opener alone did not alter the nociceptive threshold in the formalin test. It is possible that the antinociceptive effect of quinine is related to several mechanisms that could suppress pain signal transmission [14].

In this study we examined the antinociceptive effect of carbenoxolone, a gap junction blocker, using the formalin test as a model of pain. We hypothesized that if gap junction channels are important in pain facilitation and/or propagation, carbenoxolone will reduce nociception, which may suggest novel treatment strategies for pain in humans. It seems that our findings provide important information about the role of gap junction channels in the pathology of pain.

Materials and Methods

Animals
Male NMRI mice (25-30 g) were obtained from the Razi Institute (Karaj, Iran) and housed in groups of five per cage under standard laboratory conditions. Animals were kept at constant room temperature (21±2°C) under a 12h:12h light-dark regime with free access to food and water. All animal experiments were carried out in accordance with the European Communities Council directive established November 24, 1986 (86/609/EEC) so as to minimize the number of animals used and animal suffering.

Chemicals

Carbenoxolone was purchased from Sigma. Morphine was obtained from Temad Pharmaceutical Co., Iran. All drugs were dissolved in saline and prepared fresh and administered intraperitoneally (i.p.).

Antinociception recording

A 25-µl volume of 2.5% formalin was injected subcutaneously into the dorsal surface of the right hind paw of the mouse using a microsyringe with a 26-gauge needle. Immediately after formalin injection, animals were placed individually in a glass cylinder on a flat glass floor and a pain response was defined as licking or biting of the affected paw [15]. The animals were monitored for a period of 50 minutes following formalin injection for occurrence of licking responses. The first five minutes following injection is the first phase, and from time 15-50 minutes was the second phase. Pain responses during each of these phases were measured, and mean ± S.E.M. durations those animals spent licking and biting between during each phase are presented here.

Drug treatment

In the first set, five groups of animals were treated with carbenoxolone (5, 10, 20, 40 or 80 mg/kg, i.p) and one group was injected with saline 30 min before formalin injection and antinociception was assessed as described above. In the second set of animals, five groups of animals were treated with carbenoxolone (5, 10, 20, 40 or 80 mg/kg, i.p.) and one group was injected with saline. Then, within each group, animals were treated with morphine (1.5, 3, or 6 mg/kg, i.p.) 30 and 15 min respectively before formalin injection.

Statistical analyses

Analyses of variance (ANOVA), followed by Tukey-Kramer was used for all data. Statistical results with $P<0.05$ were considered significant.

Results

The antinociceptive effect of carbenoxolone on the first and second phases of the formalin test is represented in Fig 1. Administration of carbenoxolone (5-80 mg/kg, i.p.) to mice induced significant antinociception in the first phase of the formalin test (Fig.1A). Also, carbenoxolone (5-80 mg/kg) induced significant antinociception in the second phase of the formalin test ($P<0.001$) (Fig.1B).
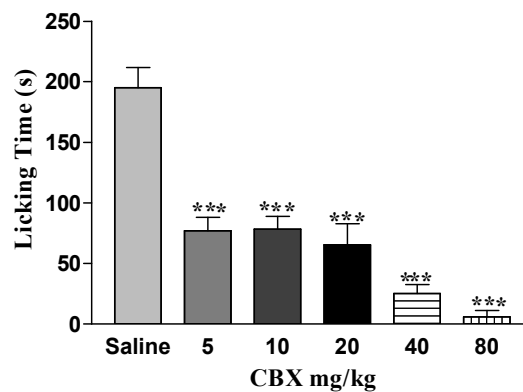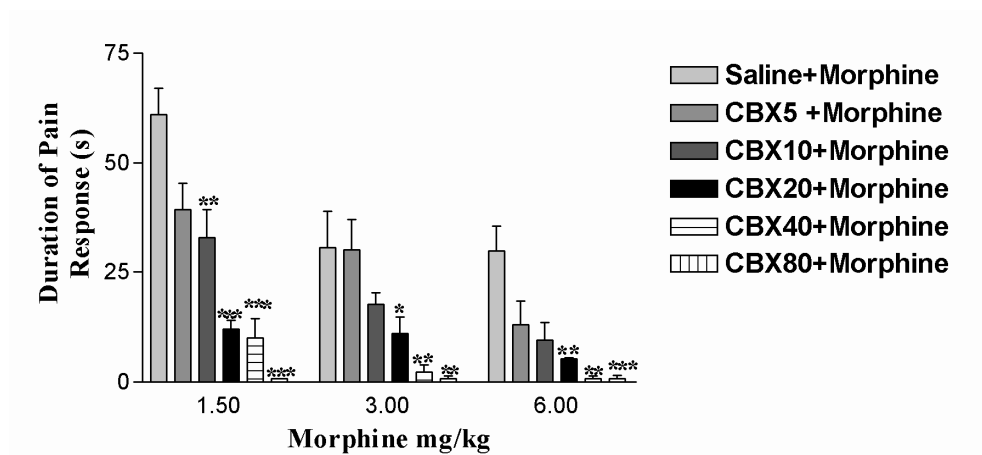
A)
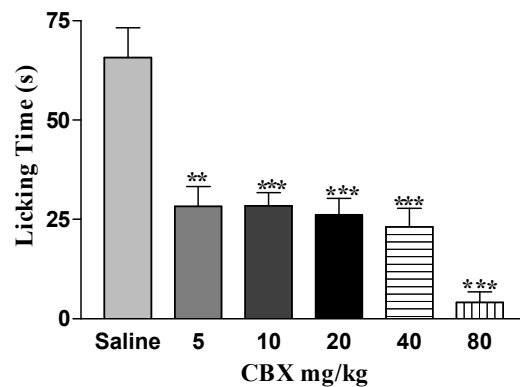
B)

Fig 1. Antinociceptive effect of carbenoxolone in the formalin test. Mice were injected with either saline (10 ml/kg, i.p.) or different doses of carbenoxolone (5, 10, 20, 40, 80 mg/kg, i.p.) 30 min before formalin injection. Duration of paw licking and biting was recorded during minutes 0-5 (panel A; first phase) and 15-50 min (panel B; second phase) after formalin injection was recorded. Each point represents the mean ± S.E.M. of 20 experiments. **$P < 0.01$, ***$P < 0.001$, compared to saline control group, Tukey-Kramer Test

The antinociceptive effect of morphine (1.5, 3 or 6 mg/kg, i.p.) in the presence or absence of carbenoxolone (5-80 mg/kg, i.p.) on the first and second phases of the formalin test is represented in Fig 2. Administration of carbenoxolone (5-80 mg/kg, i.p.) to mice significantly enhanced antinociception in the first phase of the formalin test (Fig.1A). Also, carbenoxolone (5-80 mg/kg) significantly increased antinociception of morphine at dose of 1.5 mg/kg in the second phase of the formalin test ($P < 0.001$) (Fig.1B).
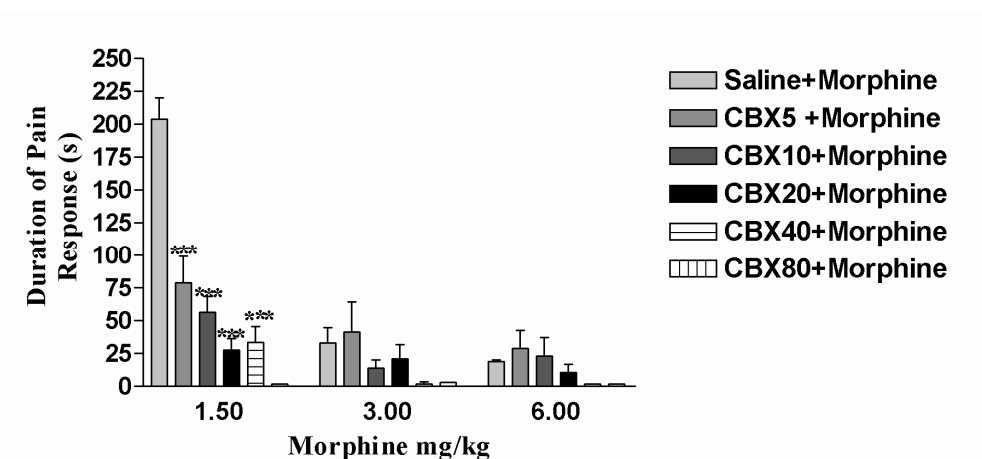
A)

B)

Fig 2. Antinociceptive effect of morphine (1.5, 3 or 6 mg/kg) in the presence or absence of carbenoxolone (5, 10, 20, 40 or 80 mg/kg) on the first and second phases of the formalin test. Mice were injected with either saline (10 ml/kg, i.p.) or different doses of carbenoxolone (5, 10, 20, 40, 80 mg/kg, i.p.) 30 min before formalin injection and morphine (1.5, 3 or 6 mg/kg) was injected 15 min after carbenoxolone injection. Duration of paw licking and biting was recorded during minutes 0-5 (panel A; first phase) and 15-50 min (panel B; second phase) after formalin injection was recorded. Each point represents the mean ± S.E.M. of 20 experiments. *P< 0.05,**P < 0.01, ***P < 0.001, compared to control group (saline+ morphine), Tukey-Kramer Test

Discussion

In the present study, the effects of carbenoxolone, a gap junction blocker, and morphine were examined using the formalin test as a model of pain. Our results indicate that carbenoxolone has dose-dependent antinociceptive properties in the first and second phases of the formalin test. Furthermore, carbenoxolone could increase the effect of morphine in the both phases of formalin test. However, this enhancing effect in the first phase was more than second phase.

Formalin produced a distinct biphasic response with respect to pain. Two distinct periods of high licking activity were identified after the formalin injection. The early phase began immediately after the injection and the late persistent phase began 20-25 min after the formalin injection. The first phase is thought to reflect direct activation of primary afferent fibers, including both low threshold mechanoreceptive and nociceptive types. The second phase reflects a facilitated state of spinal and supraspinal sensitization driven by persistent primary afferent inputs that results in release of excitatory amino acids and neuropeptides [16,17].

Our findings from the first and second phases for formalin-induced pain are similar to previous studies that have shown that carbenoxolone suppresses activated astrocytes via heterotypic gap junction channels in the formalin test and in neuropathic pain in response to sciatic nerve inflammation [13,18]. It has been shown that the activation of cutaneous C-fibers by capsaicin or sciatic nerve transection increases the number of astrocytic gap junctions as well as the expression level of connexin 43 in the dorsal horn on the stimulated side. Recently, it was shown that the number of heterotypic gap junction channels increases significantly following subcutaneous formalin injection [18]. Furthermore, it has been suggested that spinal cord glial cells may play an important role in modulation of hyperalgesia induced by formalin injection into the hind paw of rats through heterotypic gap junction channels that are located between astrocytes and neurons [19]. Thus, it is possible that gap junction blockade is involved in the antinociceptive activity of carbenoxolone. Furthermore, it is possible that other mechanism may be involved in antinociception of it. On the other hand, intrathecal pretreatment with carbenoxolone attenuated the antinociception produced by morphine in hot plate test [20]. It is possible that the role of gap junction channels in modulation of hyperalgesia in hot plate test is different than formalin. Further studies must be conducted.

Conclusion

As our supposedly hypothesis, carbenoxolone has antinociceptive effect in the both phases of formalin test. Pretreatment with carbenoxolone increased antinociceptive effect of morphine in the first phase of formalin test. Moreover, pretreatment of carbenoxolone at lower dose of morphine could enhance antinociceptive effect of it in the second phase of formalin test. . It is suggested that structure–activity studies of carbenoxolone, a gap junction channel blocker, will perhaps lead to the synthesis of a carbenoxolone based derivative that will be effective in treatment of pain in humans.

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