

The Interaction Between Quinine and Trimethylamine in the Formalin Test

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Summary

In this study, the influences of the gap junction blocker quinine and the gap junction opener trimethylamine (TMA) in antinociception were examined using the formalin test as a model of pain. We found that quinine was dose-dependently antinociceptive in both the early and late phases of the formalin test. In contrast, TMA alone did not change the nociceptive threshold in the formalin test. In the both phases of the formalin test, TMA increased antinociception of quinine. It couldn't conclude that gap junction blockade plays role in the mechanism by which quinine suppresses pain responses in the formalin test. Furthermore, it seems that pretreatment with TMA has additive effects on the antinociceptive effect of quinine in the formalin test.

Keywords: Quinine, Trimethylamine, Antinociceptive, Formalin test

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Introduction

Quinine, an anti-malaria drug, specifically blocks Cx36, and with lesser potency Cx50, in mammalian cells. Cx36 is exclusively expressed in neurons and is the principal connexin in adult neurons [1]. Trimethylamine (TMA) is a gap junction channel opener and has been reported to open gap junctions as a result of intracellular alkalinization [2-4]. Previous works have shown that gap junction openers exacerbate seizure activity [5-7]. Quinine (a tertiary amine, $pK_a \approx 8.7$) has been shown to close gap junction channels in a reversible, concentration-dependent and connexin-specific manner. Furthermore, the binding site was found to be intracellular and likely coincides with that for local anesthetic molecules [1].

Based on similar properties between quinine and local anesthetic molecules, in this study we examined the antinociceptive effect of quinine using the formalin test as a model of pain. We hypothesized that if gap junction channels are important in pain facilitation and/or propagation, quinine will reduce nociception, which may suggest novel treatment strategies for pain in humans. No previous studies have examined the ability of gap junction openers to potentate pain, so we also studied the effect of TMA on nociception. Together, 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 ($21 \pm 2^\circ\text{C}$) 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

Quinine anhydrous hydrochloride was purchased from Fluka, and TMA was obtained from Sigma. Quinine was dissolved in 0.8% (v/v) Tween 80. All drugs were 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 [8]. 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 \pm S.E.M. durations those animals spent licking and biting between during each phase are presented here.

Drug treatment

Three sets of animals were used for these experiments. In the first set, six groups of animals were treated with quinine (2, 5, 10, 20, 40 or 80 mg/kg, i.p) 30 min before formalin injection and antinociception was assessed as described above. In the second set of animals, three groups of mice were treated with TMA (0.1, 1 or 10 mg/kg, i.p.) 40 min before formalin injection. In the third set, eight groups of animals were treated with TMA (1 or 10 mg/kg, i.p.) and within each group, animals were treated with quinine (10, 20, 40 or 80 mg/kg, i.p.) 40 and 30 min respectively before formalin injection.

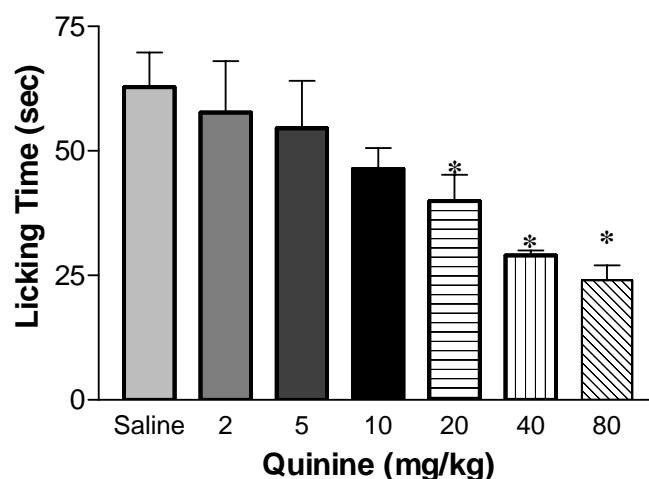
Statistical analyses

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 quinine on the first and second phases of the formalin test is represented in Figure 1. Administration of quinine (10 or 80 mg/kg, i.p.) to mice induced significant antinociception in the first phase of the formalin test ($P < 0.05$) (Fig 1A). Also, quinine (10-80 mg/kg) induced significant antinociception in the second phase of the formalin test ($P < 0.001$) (Fig 1B).

A)



B)

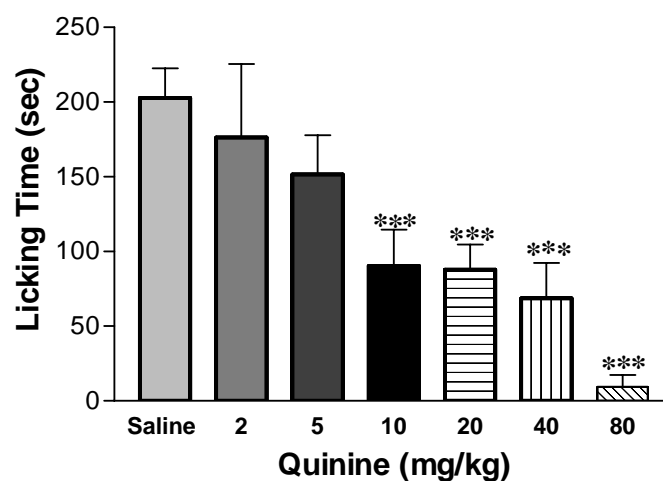
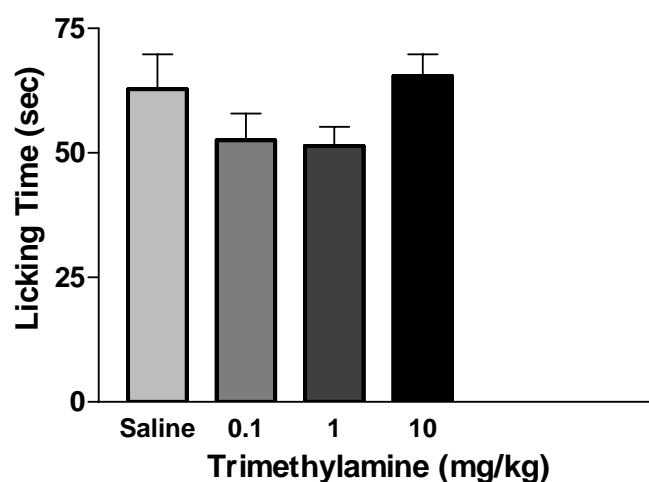


Fig 1. Antinociceptive effect of quinine in the formalin test. Mice were injected with either saline (10 ml/kg, i.p.) or different doses of quinine (2, 5, 10, 20, 40, 80 mg/kg, i.p.) 30 min before formalin injection. Duration of paw licking and biting was recorded during minutes 0-5 (panel A; first phase) and 15-50 min (panel B; second phase) after formalin injection was recorded. Each point represents the mean \pm S.E.M. of 20 experiments. * $P < 0.05$, *** $P < 0.001$ for quinine versus respective saline control group, Tukey-Kramer Test

The effect of TMA in the formalin test is represented in Fig 2. Various doses of TMA (0.1, 1 or 10 mg/kg, i.p.) produced no analgesia in either phase of the test (Fig 2A, B).

A)



B)

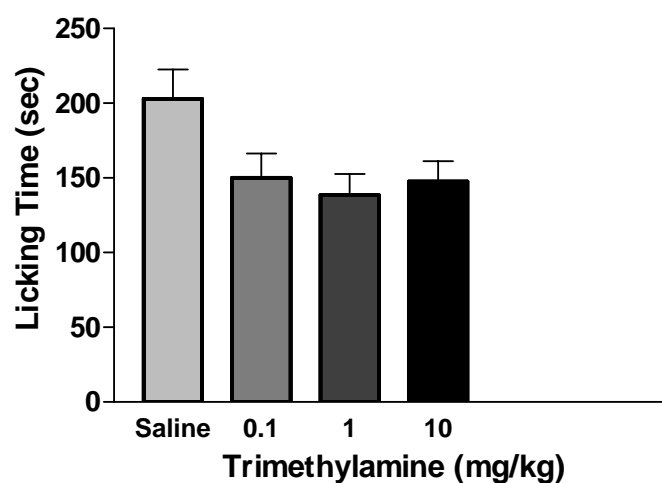


Fig 2. Effect of TMA in the formalin test. Mice were injected either with saline (10 ml/kg, i.p.) or different doses of TMA (0.1, 1 and 10 mg/kg, i.p.) 40 min before formalin injection. Antinociception during minutes 0-5 (panel A; first phase) and 15-50 min (panel B; second phase) after formalin injection was measured. Each point represents the mean \pm S.E.M. of 20 experiments compared to control group, Tukey-Kramer Test

Table 1, show the effect of quinine (10, 20, 40 or 80 mg/kg) in the presence or absence of TMA (1 or 10 mg/kg) on the first and second phases of the formalin test. There were no significant different between coadministration quinine (10, 20, 40 or 80 mg/kg) and TMA (1 or 10 mg/kg) for the first and second phases in the formalin test ($P > 0.05$) (Table1).

Table 1. Effect of quinine in the presence of saline, or TMA in the formalin test

Treatment	Quinine 10mg/kg	Quinine 20mg/kg	Quinine 40mg/kg	Quinine 80mg/kg
A				
Saline	45.4 \pm 4.2	39.9 \pm 5.3	29 \pm 0.8	24 \pm 6
TMA 1mg/kg	53.5 \pm 5.7	35.8 \pm 8.1	37.8 \pm 9	26 \pm 8.5
TMA 10mg/kg	35.5 \pm 5.3	32.1 \pm 5.1	39 \pm 5	18.9 \pm 4.7
B				
Saline	90.4 \pm 24.2	87.7 \pm 17	68.6 \pm 23.8	9.4 \pm 7.9
TMA 1mg/kg	102.4 \pm 35.7	81.7 \pm 22	38 \pm 19.2	4.2 \pm 2.8
TMA 10mg/kg	147.4 \pm 49.4	87.7 \pm 13.2	23 \pm 9.8	8 \pm 3.8

TMA (1 or 10 mg/kg) and quinine (10, 20, 40 and 80 mg/kg) were respectively administrated 40 and 30 min prior to formalin test in mice. Duration of paw licking and biting was recorded during minutes 0-5 (A: first phase) and 15-50 min (B: second phase) after formalin injection. Each point represents the mean \pm S.E.M of 20 experiments.

Discussion

In the present study, the effects of quinine, a gap junction blocker, and TMA, a gap junction opener, were examined using the formalin test as a model of pain. Our results indicate that quinine 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, TMA alone did not alter the nociceptive threshold in the formalin test.

In addition to studying the effect of each gap junction modulator alone, we looked at the effect co-administration of quinine and TMA with the hypothesis that TMA would reduce the antinociceptive effect of quinine. Despite of supposedly hypothesis, TMA has increased the effect of quinine in the both phases of formalin test. Therefore, it is possible that the two drugs might share a common mechanism during the both phases that is evident with high doses of quinine and TMA.

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 [9,10].

Recently, we found that TMA could reverse the anticonvulsant effect of quinine in the pentylenetetrazole (PTZ) model in rats [11]. These findings indicated that the two drugs work through gap junction channels. Furthermore, in our previous study, the effect of TMA on generalized tonic-clonic seizure (GTCS) decreased with increasing dose in the PTZ model [11]. Despite of supposedly contrasting mechanism of action, TMA has potentiated the effect of quinine in the two phases of formalin test. Thus, it is possible that other mechanisms than gap junction blockade are involved in the antinociceptive activity of quinine. These possible mechanisms are e.g. potassium channel blockade and curare-like effects decreasing the excitability of the skeletal muscle and increasing the refractory period following the use of quinine. On the other hand, in 1992, Amabeoku *et al* have reported such an effect. Influence of some dopaminergic agents on antinociception produced by quinine in mice. In that report, the dopaminergic mechanism was suggested to be involved in the analgesic effect of quinine [12]. In other words, it is possible that the antinociceptive effect of quinine is related to several mechanisms that could suppress pain signal transmission, but further studies must be conducted to validate this proposal.

Conclusion

The present study provides evidence for the antinociceptive effect of quinine in the formalin test. The effect of quinine was apparent in the first phase, but was more prominent in the second phase following formalin injection, which provides insight into the mechanism of each phase and, in turn, identification of effective therapeutic agents. In the view of above, we suggest that the antinociceptive effect of quinine action is not related to gap junction blockade. Structure-activity studies of quinine will perhaps lead to the synthesis of their effective derivatives for the control of pain.

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