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The role of 5-HT$_3$ receptors in the additive anticonvulsant effects of citalopram and morphine on pentylenetetrazole-induced clonic seizures in mice

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ABSTRACT

Citalopram, a selective serotonin reuptake inhibitor (SSRI), is frequently used in the treatment of major depressive disorders. In addition to its antidepressant features, citalopram shows some anticonvulsive properties at lower doses, whereas higher doses, ingested in cases of suicide, have been associated with seizures. Moreover, some reports support the enhancing effect of morphine on different responses of SSRIs such as analgesic and anticonvulsant properties. Although the exact mechanisms of these additive effects are not yet fully understood, 5-HT$_3$ receptor has recently been shown to play an important role in the central effects of SSRIs and morphine. In this regard, we used a model of clonic seizures induced by pentylenetetrazole (PTZ) in male NMRI mice to investigate whether morphine and citalopram exhibit additive anticonvulsant effects and, if so, whether this effect is mediated through modulation of 5-HT$_3$ receptors. In our study, citalopram at lower doses (0.5 and 1 mg/kg, ip) significantly increased the seizure threshold ($P<0.01$) and at a higher dose (50 mg/kg) had proconvulsive effects. Moreover, morphine at low and noneffective doses had additive effects on the anticonvulsive properties of citalopram. This additive effect was prevented by pretreatment with low and noneffective doses of tropisetron (a 5-HT$_3$ receptor antagonist) and augmented by 1-(*m*-chlorophenyl)-biguanide (mCPBG, a 5-HT$_3$ receptor agonist). Moreover, low doses of morphine (0.1 and 0.5 mg/kg) alone or in combination with potent doses of 5-HT$_3$ receptor agonist or antagonist could not alter the proconvulsive properties of citalopram at higher dose (50 mg/kg), ruling out the contribution of 5-HT$_3$ to this effect. In summary, our findings demonstrate that 5-HT$_3$ receptor mediates the additive anticonvulsant properties of morphine and low-dose citalopram. This could constitute a new approach to augmenting the efficacy and curtailing the adverse effects of citalopram.

1. Introduction

Citalopram, a selective serotonin reuptake inhibitor (SSRI), is broadly used in the treatment of major depressive disorders [1,2]. Drugs in this class enhance serotonergic neurotransmission through potent and selective inhibition of serotonin reuptake in neuronal synapses [1,2]. In addition to its therapeutic effects, a handful of animal studies report that citalopram, like other SSRIs, possesses anticonvulsive properties at lower doses [3,4]. Some clinical trials also state that citalopram is a safe and effective antidepressant in the treatment of depressed patients with epilepsy [5]; in one study, chronic treatment with citalopram was associated with an improvement in depressive symptoms and reduction in seizure frequency in patients [6]. Several lines of evidence indicate that a serotonergic deficit may be involved in epileptogenesis, and therefore, serotonin demonstrates protective characteristics in the central nervous system (CNS) [4,7]. In contrast, higher doses of citalopram (deliberately used in suicide) can cause seizures [8,9]. Generalized seizures occur in 2 to 6% of patients who present to the hospital following deliberate citalopram overdose [10,11]. Regarding these paradoxical effects on seizure, no clear mechanism has been yet established.

We previously characterized morphine as having a biphasic modulatory effect on seizure threshold in different seizure paradigms, where low doses produce an anticonvulsant effect and high doses promote convulsions [12,13]. In addition, neuromodulatory interaction between the opioid and serotonergic systems in the brain has been the subject of many studies; some data show that combinations of serotonergic antidepressants and opioid receptor agonists may exert additive antidepressant [14] or antinociceptive [15] effects. Likewise, fluoxetine has a potentiating effect on the antinociceptive effect of morphine [16,17]. Recently, the 5-HT$_3$ receptor has received much attention for its possible contribution to many central responses to morphine [18,19]. The 5-HT$_3$ receptor has also been shown to be specifically involved in a number of citalopram's central properties [20].

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Considering the functional interaction between the serotonergic and opioid systems in the CNS and the newly discovered implication of the 5-HT_3 receptor in several central responses of morphine and citalopram, we designed this study to examine whether the concurrent administration of SSRIs and morphine enhances their anticonvulsant features. Using 1-(*m*-chlorophenyl)-biguanide (a 5-HT_3 receptor selective agonist) and tropisetron (a 5-HT_3 receptor selective antagonist), we examined the possible involvement of 5-HT_3 receptors in the additive anticonvulsant effects of citalopram and morphine co-administration on threshold to pentylenetetrazole-induced clonic seizures in mice.

2. Materials and methods

2.1. Animals

Male NMRI mice weighing 23–30 g (Razi Institute, Karadj, Iran) were used in the study. The animals were housed in standard polycarbonate cages in groups of four or five in a temperature-controlled room (22 °C) with a 12-hour-light/12-hour-dark cycle. Animals were acclimated at least 2 days before experiments with free access to food and water. Experiments were conducted between 09:00 and 15:00 hours. All procedures were carried out in accordance with institutional guidelines for animal care and use, and all possible measures were taken to minimize the number of animals used and their suffering, including immediate euthanasia after acute experiments. Groups consisted of at least eight animals and each animal was used only once.

2.2. Drugs

The following drugs were used throughout: pentylenetetrazole (PTZ), morphine sulfate (Sigma, UK), *m*-chlorophenylbiguanidine (mCPBG, a 5-HT_3 receptor selective agonist), and tropisetron (a 5-HT_3 receptor selective antagonist) (Sigma–Aldrich, UK). Citalopram was a generous gift from Bakhtar Bioshimi (Kermanshah, Iran). Injections were done in the volume of 10 mL/kg. All drugs were dissolved in normal saline and administered intraperitoneally. To assess clonic seizures, PTZ was administered intravenously (0.5%).

The doses were chosen on the basis of previously published studies [13,21] and pilot experiments. Citalopram was injected 15 minutes after 5-HT_3 receptor agonist or antagonist and 30 minutes before performing the test. In this manner, tropisetron would have enough time to block the receptors and mCPBG would have enough time to exert its effect. The results suggest that the selected time intervals allowed effective activation or blockade of 5-HT_3 receptors.

2.3. Seizure paradigms

The clonic seizure threshold was determined by inserting a 30-gauge dental needle into the lateral tail vein of the mouse [13,22]. The needle was then secured in place with a narrow piece of adhesive tape. With the mouse moving freely, the PTZ solution (0.5%) was slowly infused into the tail vein at the constant rate of 1 mL/minute using an infusion pump (NE 1000, New Era Pump System, Inc) connected to the dental needle by a polyethylene tube. Infusion was halted when general clonus (forelimb clonus followed by full clonus of the body) was observed. The minimal dose of PTZ (mg/kg mouse wt) needed to induce general clonus was recorded as an index of clonic seizure threshold. As such, seizure threshold is time related and dependent on the PTZ dose administered.

2.4. Experiments

In experiment 1, different doses of citalopram (0.1, 0.5, 1, 5, 10, 25, and 50 mg/kg) were injected 30 minutes prior to the determination of threshold to clonic seizures induced by intravenous administration of PTZ solution. Control animals received the same volume of isotonic saline in all experiments. The doses and time point were chosen on the basis of pilot studies. In experiment 2, morphine was injected at different doses (0.1, 0.5, 1, 10, 15, and 30 mg/kg) 30 minutes before determination of seizure threshold; the corresponding control group received saline at the same time point. In experiment 3, subeffective doses of morphine (0.1 and 0.5 mg/kg) were administered concomitantly with low doses of citalopram (0.1 and 0.5 mg/kg) to examine the effect of these combinations on seizure threshold. In experiment 4, mCPBG (1 and 5 mg/kg) was injected alone and 15 minutes before the subeffective combinations of morphine (0.1 mg/kg) and citalopram (0.1 and 0.5 mg/kg) and 45 minutes before determination of seizure threshold; the corresponding control group received saline at the same time. In experiment 5 animals received acute intraperitoneal injections of tropisetron (0.25 and 2 mg/kg) alone or 15 minutes before effective combinations of morphine (0.5 mg/kg) and citalopram (0.1 and 0.5 mg/kg). In experiment 6, animals received a high dose of citalopram (50 mg/kg) with noneffective doses of morphine (0.1 and 0.5 mg/kg). Then selected doses of mCPBG (5 mg/kg) or tropisetron (2 mg/kg) were injected before a combination of citalopram (50 mg/kg) and morphine (0.5 mg/kg). The 5-HT_3 receptor agonist or antagonist was injected 45 minutes, and citalopram and morphine 30 minutes, before determination of seizure threshold.

2.5. Statistical analysis

Seizure thresholds are expressed as the mean ± SEM clonic seizure threshold in each experimental group. One-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparisons was used for data analysis. Two-way ANOVA was employed to analyze the effect of combinations of citalopram with various doses of morphine. In all experiments, a P value of 0.05 was considered the level of significance.

3. Results

3.1. Effect of different doses of citalopram on seizure threshold

Fig. 1 illustrates the biphasic effect of acute intraperitoneal administration of different doses of citalopram (0.1, 0.5, 1, 5, 10, 25, and 50 mg/kg, ip) on threshold to PTZ-induced clonic seizures. Seizure

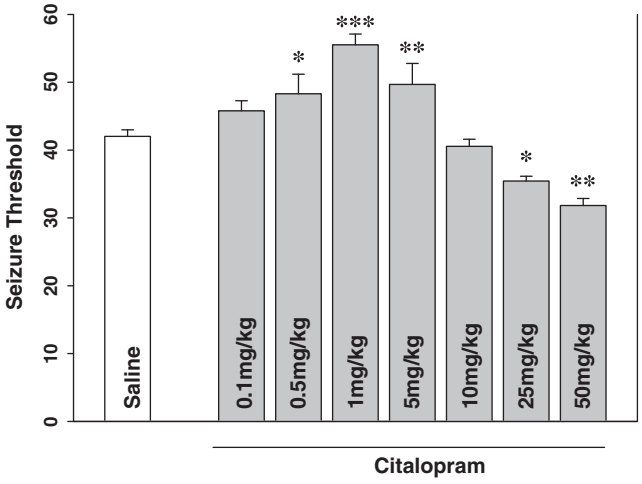

Fig. 1. Effect of administration of different doses of citalopram (0.1, 0.5, 1, 5, 10, 25, and 50 mg/kg, ip) on threshold to PTZ-induced seizures in mice. Citalopram was administered 30 minutes before determination of PTZ seizure threshold. Data are expressed as the mean ± SEM seizure threshold in each group. Each group consisted of at least eight mice. *P<0.05, **P<0.01, ***P<0.001, compared with saline control group.