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5-HT_3 receptor mediates the dose-dependent effects of citalopram on pentylenetetrazole-induced clonic seizure in mice: Involvement of nitric oxide

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Summary Citalopram is a selective serotonin reuptake inhibitor (SSRI), widely used in the treatment of depressive disorders. It has been shown that citalopram affects seizure susceptibility. Although the exact mechanism of these effects are not yet fully understood, recent data suggest that $5HT_3$ receptors and nitric oxide (NO) might participate in the central effects of SSRIs. In this study in a mouse model of clonic seizure induced by pentylenetetrazole we investigated whether 5-HT_3 receptors are involved in the effects of citalopram on seizure threshold. In our experiments, citalopram at lower doses (0.5 and 1 mg/kg, i.p) significantly increased the seizure threshold and at higher doses ($\geq$25 mg/kg) showed proconvulsive effects. Moreover, mCPBG (a 5-HT_3 receptor agonist) at low and non-effective doses augmented while non-effective doses of tropisetron prevented the anticonvulsive properties of citalopram.

On the other hand, Low doses of nitric oxide synthase inhibitors L-NAME and 7-NI alone or in combination with lower doses of 5-HT_3 receptor agonist enhanced the anticonvulsive property of citalopram, while L-arginine (NO precursor) alone or in combination with tropisetron blocked the protective effect of citalopram.

In summary, our findings demonstrate that 5-HT_3 receptor mediates the anticonvulsant properties of low doses of citalopram, whereas it seems that the proconvulsive effect is mostly mediated through the NO pathway and can be totally blocked by NOS inhibitors. This could propose a new approach toward finding the mechanism of citalopram activity, curtailing the adverse effects of citalopram and perhaps managing the convulsions as a vicious consequence of citalopram overdose.

Abbreviations: NO, nitric oxide; NOS, nitric oxide synthase; mCPBG, meta-Chlorophenylbiguanidine; L-NAME, NG-nitro-L-arginine methyl ester; 7-NI, 7-nitroindazole.

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Introduction

Citalopram is a selective serotonin reuptake inhibitor (SSRI) mainly used in the treatment of major depressive disorder (Baumann, 1992; Price, 1999). In addition to its antidepressant features, some anticonvulsant and antiepileptic properties have been reported for citalopram in different animal models and clinical trials (Kabuto et al., 1994; Favale et al., 2003; Clinckers et al., 2004a). In contrast, seizure is an adverse effect of high doses of citalopram when ingested accidentally or intentionally (Fisher et al., 2002; Waring et al., 2008). In fact, these biphasic dose-dependent properties of antidepressants including SSRIs have been the subject of many studies (Loscher, 2009). However, no clear mechanism for these paradoxical responses of citalopram on seizures has been yet established.

Regarding the possible pathways involved in SSRIs effects; different subtypes of serotonin receptors including $5HT_{1A}$, $5HT_2$ and $5HT_3$ receptors have received much attention for their potential contribution to many central responses of SSRIs (Eison and Mullins, 1995; Stahl, 1998; Keltner et al., 2002). It is now obvious that many SSRIs including citalopram exert some of their pharmacological effects such as antidepressant activity via serotonin type 3 receptors (5-HT_3) (Fan, 1994; Redrobe and Bourin, 1997; Choi et al., 2003). Moreover some important adverse effects of SSRIs including hyperglycemia (Carvalho et al., 2004), sexual dysfunction (Nelson et al., 1997) and nausea (Limebeer et al., 2009) might be mediated through 5-HT_3 receptor activation.

5-HT_3 receptor is the only ligand-gated ion channel among seven known classes of serotonin receptors (Derkach et al., 1989). This receptor plays an important role in regulating communication between cells in the central and peripheral nervous systems and is the target of many different therapeutic agents and abused drugs (Derkach et al., 1989; Chameau and van Hooft, 2006). Recently, the possible participation of nitric oxide (NO) in many functional responses of 5-HT_3 receptor activation has been investigated. For instance, NO contributes to the pressor effect elicited by 5-HT_3 receptor stimulation in the nucleus tractus solitarii (Sevoz-Couche et al., 2002) and neurogenic relaxations of proximal colon in guinea pig (Riad et al., 1994). In this regard, lately, we have shown that 5-HT_3 receptor plays an important role in seizure susceptibility of mice in PTZ-induced seizure model and investigate role of NO as a modulator in this phenomenon (Gholipour et al., 2010; Bahremand et al., 2011).

The exact mechanisms by which serotonin 5-HT_3 receptor interacts with nitrergic system in neuronal cells is not totally understood, though different possible mechanisms like modulatory effect on cytosolic Ca^{2+} activity due to entry of extracellular Ca^{2+} have been proposed (Reiser, 1990).

In the present study we have investigated the dose-dependent effects of citalopram on seizure susceptibility. Using a selective 5-HT_3 receptor agonist (mCPBG) and antagonist (Tropisetron), we also examined the potential role of 5-HT_3 receptors. Finally, involvement of 5-HT_{1A} receptors was assessed by means of WAY-100635, a selective 5-HT_{1A} antagonist. We examined the modulatory effects of NO and how this contributed to the dose-dependent properties of citalopram.

Materials and methods

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 4—5 and they were in a temperature-controlled room (22 C) with 12 h light/12 h dark cycle. Animals were acclimated at least 2 days before experiments with free access to food and water. The experiments were conducted between 09:00 and 15:00. All procedures were carried out in accordance with institutional guidelines for animal care and use and possible measures were undertaken to minimize the number of animals used and also to minimize animals' discomfort including immediate euthanasia after acute experiments. Groups consisted of at least eight animals and each animal was used only once. Additionally, all efforts were made to reduce animal suffering and to use only the number of animals necessary to produce reliable scientific data.

Drugs

The following drugs were used throughout the study: Pentylenetetrazole (PTZ) (Sigma, UK), L-arginine (L-ARG), NG-L-arginine methyl ester (L-NAME), 7-nitroindazole (7-NI) (Sigma, St Louis, MO, USA), WAY-100635, m-Chlorophenylbiguanidine (mCPBG) and Tropisetron (Sigma—Aldrich, UK). Citalopram was a generous gift from Bakhtar shimi (Kermanshah, Iran). WAY-100635 administered subcutaneously (s.c) and all other drugs, except PTZ, were administered intraperitoneally (i.p), injected in a volume of 10 ml/kg of the body weight of the mice. 7-NI was suspended in a 1% aqueous solution of tween 80 and all other drugs were dissolved in normal saline. To provoke clonic seizures, PTZ was infused intravenously (0.5%, i.v.) into the lateral tail vein of mouse (see Section ''Seizure paradigms'').

The doses were chosen based on previously published studies (Bahremand et al., 2010; Gholipour et al., 2010) and pilot experiments. In experiments with sequential treatments, the intervals between NOS inhibitors or L-ARG and the agonist or antagonist of $5HT_3$ were 15 min so the effective blockade of enzymes by inhibitors were allowed. Citalopram was injected 15 min after administration of $5HT_3$ agonist or antagonist or $5HT_{1A}$ antagonist i.e. 30 min before performing the test. As such, tropisetron or WAY-100635 was given enough time to blockade the receptors before injects the citalopram and also mCPBG to exerts its effect. The results are suggestive that selected time intervals allowed effective activation or blockade of receptors.

Seizure paradigms

The clonic seizure threshold was determined by inserting a 30-gauge dental needle into the lateral tail vein of mouse (Loscher, 2002). The needle was then secured to the tail by a narrow piece of adhesive tape. With mouse moving freely, the PTZ solution (0.5%) was slowly infused into the tail vein at a constant rate of 1 ml/ min using an infusion