European Journal of Pharmacology 791 (2016) 369–376

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Behavioural pharmacology

The effect of nitrazepam on depression and curiosity in behavioral tests in mice: The role of potassium channels

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ARTICLE INFO

Article history:
Received 29 July 2016
Received in revised form
6 September 2016
Accepted 7 September 2016
Available online 9 September 2016

Keywords:
Nitrazepam
Potassium channel
Forced swimming test
Tail suspension test
Hole-board test
Mice

ABSTRACT

Evidence show that gamma-aminobutyric acid (GABA) receptors are involved in depression, so the aim of this study was to investigate the effect of nitrazepam as agonist of $GABA_A$ receptors on depression and curiosity in male mice and the role of potassium channel in antidepressant-like response. For this purpose, we studied the antidepressant-like properties of fluoxetine, nitrazepam, glibenclamide, and cromakalim by both forced swimming test (FST) and tail suspension test (TST). Animals were injected by various doses of nitrazepam (0.05, 0.1, and 0.5 mg/kg). Nitrazepam at dose of 0.5 mg/kg significantly decreased the immobility time compared to control group in both FST and TST. Fluoxetine also showed such a response. Co-administration of nitrazepam (0.05 mg/kg) with glibenclamide in TST (1 mg/kg) and in FST (0.3, 1 mg/kg) also showed antidepressant-like response. Beside, cromakalim (0.1 mg/kg) could reverse the antidepressant-like effect of nitrazepam (0.5 mg/kg) in both FST and TST, while cromakalim and glibenclamide alone could not change the immobility time compared to control group ($P > 0.05$). The hole-board test revealed that nitrazepam at doses of 0.5 and 0.1 mg/kg could increase the activity of the animal's head-dipping and boost the curiosity and exploration behavior of mice. The results of this study revealed that nitrazepam may possess antidepressant-like properties and this effect is dependent to potassium channels in both FST and TST.

1. Introduction

Depression is a mood disorder that causes a persistent feeling of sadness and is a major cause of suicides (Burt and Stein, 2002; McCall et al., 2002). Depressive disorders are usually associated with dramatic functional impairment and health care cost (Brown et al., 2004). In view of the fact that the prevalence of depression is progressively high (Compton et al., 2006), increasing attention is paid to treat patients suffering from depression. Amongst the several routine prescribed antidepressant medications, which are mainly based on monoamine regulation, few are highly expected to show the desired outcomes (Arroll et al., 2005; Berton and Nestler, 2006). Thus, finding new antidepressants with favorable pharmacological properties is advantageous.

Evidences from previous studies confirmed the implication of gamma-aminobutyric acid (GABA) system in the pathophysiology of depressive disorders (Brambilla et al., 2003). GABAergic receptors are consist of two principal kinds with diverse distribution on the surface of neurons, $GABA_A$ and $GABA_B$ receptors. Benzodiazepines (BDZs) are agonists of $GABA_A$ receptor. These drugs cause an allosteric (structural) modification of the receptor that results in elevation of $GABA_A$ receptor activity. BDZs facilitate the action of GABA in increasing the frequency of chloride channel

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http://dx.doi.org/10.1016/j.ejphar.2016.09.017
0014-2999/

opening events, which results in elevation of chloride ion conductance and inhibition of the action potential (Donoghue and Lader, 2010). In the other hand, the stated effectiveness of BDZs in controlling of acute mood complaints is reliable with the theory of a GABAergic insufficiency in mood disorders. Clonazepam and lorazepam have definitely been recommended for treatment of manic patients (Wolfsperger et al., 2007). It is also reported that clonazepam and alprazolam are effective in management of patients suffering from bipolar and unipolar depression (Dunner et al., 1987; Jonas and Cohon, 1993; Kishimoto et al., 1988; Rush et al., 1984).

Nitrazepam is a BDZ, which is prescribed in treatment of severe anxiety and insomnia. This drug also possess anticonvulsant and skeletal muscle relaxant properties (Yasui et al., 2005). Previously it was reported that some $GABA_A$ agonists have antidepressant-like effect (Alev and Kulkarni, 1989; Evangelista et al., 1987; Flugy et al., 1992). For instance, muscimol, a GABA agonist, is shown to decrease the immobility time, while picrotoxin, a GABA antagonist, could reverse the muscimol-induced reduction of the immobility time in mice forced swimming test (FST) (Poncelet et al., 1987). Furthermore, it is reported that some BDZs such as diazepam boosted the curiosity and exploration behaviors in animals (Barbui et al., 2011; Morishita, 2009).

Nowadays various animal models of depression based mainly on the efficacy of typical antidepressant drugs or responses to stress have been developed. FST and TST (tail suspension test) are two common behavioral models for assessment of antidepressants activities (Porsolt et al., 1977; Steru et al., 1985). Furthermore, it is well-known that drugs with potential antidepressant properties decrease the immobility time in rodents (Haj-Mirzaian et al., 2016a; Lucki, 1997; Ostadhadi et al., 2016c, 2016d, 2016e). In regard to interaction of GABA system and potassium channels (Chan et al., 2007; Lee et al., 2010), the aim of the present study was to investigate the antidepressant-like properties of nitrazepam in mice FST and TST and involvement of potassium channel in this response.

2. Materials and methods

2.1. Drugs

The following drugs were used in the study: nitrazepam, a selective agonist of $GABA_A$ receptors, glibenclamide, a K_{ATP} channel blocker, and cromakalim, a K_{ATP} channel opener, Dimethyl sulfoxide (DMSO) as glibenclamide and cromakalim vehicle, and fluoxetine, an antidepressant with selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitor activity (SSRI). All drugs were purchased from Sigma-Aldrich (St. Louis, MO, USA). The drugs were prepared freshly immediately before use. Fluoxetine and nitrazepam were dissolved in normal saline, while glibenclamide and cromakalim were dissolved in DMSO (1%). All drugs were injected intraperitoneally (i.p.), in a constant volume of 5 ml/kg body weight.

2.2. Animals

Male Naval Medical Research Institute (NMRI) mice (weighting 25–30 g) were provided from the Department of Pharmacology (Tehran University of Medical Sciences). Animals had free access to standard rodent chow and water, with a light/dark cycle of 12 h, at a temperature of 22 ± 2 °C and 80% humidity. All animal procedures were in accordance with Guide for the Care and Use of Laboratory Animals (NIH US publication, No. 85-23, revised 1985) recommendations.

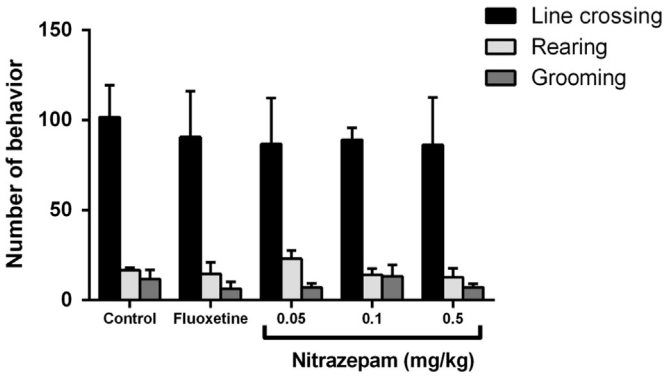

Fig. 1. Effect of acute administration of fluoxetine (20 mg/kg) and different doses of nitrazepam on number of line crossing, rearing, and grooming in open field test. Each group consists of eight mice. Values are expressed as mean+S.E.M.

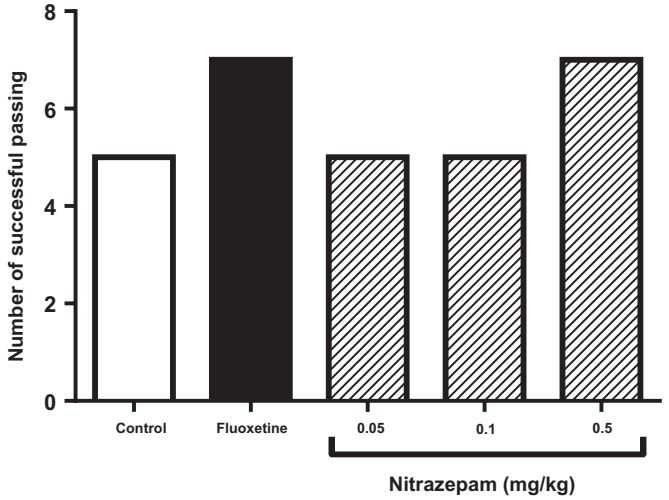

Fig. 2. Effect of acute administration of fluoxetine (20 mg/kg) and different doses of nitrazepam on number of animals with successful passing in chimney test. Each group consists of eight mice.

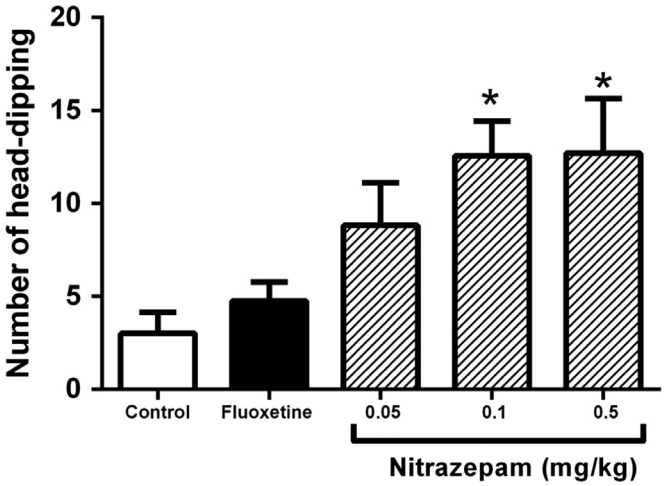

Fig. 3. Effect of acute administration of nitrazepam and fluoxetine (20 mg/kg) on number of head-dipping as a parameter of curiosity and exploration behaviors in hole-board test. Each group consists of eight mice. Values are expressed as mean ± S.E.M. $^*P < 0.05$ compared to control group.

2.3. Open field test

The OFT was carried out to measure locomotor activity in animals (Nazari et al., 2016; Ostadhadi et al., 2016b). In this common