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Research report

Selective corticotropin-releasing factor 1 receptor antagonist E2508 has potent antidepressant-like and anxiolytic-like properties in rodent models

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HIGHLIGHTS

- E2508 is a newly discovered orally active CRF_1 receptor antagonist.
- A single dose of E2508 shortened immobility time in the rat forced swim test.
- E2508 significantly reduced anxiety-like behavior in the rat defensive burying test.
- E2508 did not affect spontaneous locomotor activity and muscle strength in mice.
- More than 7 days of fluoxetine treatment decreased sexual behavior in male rats.

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ABSTRACT

Corticotropin-releasing factor (CRF) is a hormone secreted by the hypothalamus in response to stress, and CRF antagonists may be effective for the treatment of stress-related disorders including major depressive and anxiety disorders. Here, we investigated the *in vivo* pharmacological profile of *N*-cyclopropylmethyl-7-(2,6-dimethoxy-4-methoxymethylphenyl)-2-ethyl-*N*-(tetrahydro-2*H*-pyran-4-ylmethyl)pyrazolo[1,5-*a*]pyridin-3-amine tosylate (E2508), a recently synthesized, orally active CRF_1 receptor antagonist. Oral administration of a single dose of E2508 (3 or 10 mg/kg), but not fluoxetine (30 mg/kg), a selective serotonin reuptake inhibitor (SSRI), significantly shortened immobility time in rats in the forced swim test. E2508 (10, 30, or 100 mg/kg) also showed an antidepressant-like effect in the forced swim test in mice, with no sedative or muscle relaxant effects for doses up to 100 mg/kg. Moreover, E2508 (5 or 20 mg/kg) significantly reduced anxiety-like behavior in the rat defensive burying test. Diazepam, a benzodiazepine anxiolytic agent, also showed an anxiolytic effect in the defensive burying test at the same dose that induced a muscle relaxant effect in mice. Administration of E2508 (30 mg/kg) for 14 consecutive days did not affect sexual behavior. By contrast, fluoxetine (30 mg/kg) administration for ≥7 consecutive days decreased sexual behavior.

Abbreviations: ACTH, adrenocorticotropic hormone; CRF, corticotropin-releasing factor; E2508, *N*-cyclopropylmethyl-7-(2,6-dimethoxy-4-methoxymethylphenyl)-2-ethyl-*N*-(tetrahydro-2*H*-pyran-4-ylmethyl)pyrazolo[1,5-*a*]pyridin-3-amine tosylate; HPA, hypothalamic-pituitary-adrenal; IBS, irritable bowel syndrome; MDD, major depressive disorder; R121919, 3-[6-(dimethylamino)-4-methyl-pyrid-3-yl]-2,5-dimethyl-*N*,*N*-dipropyl-pyrazolo[2,3-*a*]pyrimidin-7-amine; SSRI, selective serotonin reuptake inhibitor.

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These results indicate that E2508 has both potent antidepressant-like and anxiolytic-like effects in rodent models, and is well tolerated compared with a commonly prescribed therapeutic SSRI or benzodiazepine.

1. Introduction

Antidepressants are one of the most widely prescribed medicines in many countries. The most important classes of antidepressants are the selective serotonin reuptake inhibitors (SSRIs), serotonin-noradrenaline reuptake inhibitors, tricyclic antidepressants, and monoamine oxidase inhibitors. The main indication for use of these antidepressants is major depressive disorder (MDD). However, antidepressants are frequently used in the treatment of many other diseases including generalized anxiety disorder, panic disorder, obsessive-compulsive disorder, sleep disorder, neuropathic pain, irritable bowel syndrome (IBS), eating disorder, migraine, and attention-deficit/hyperactivity disorder. Moreover, although these medicines are clinically important, they are not ideal because of insufficient efficacy, slow onset of action, adverse effects (such as sexual dysfunction, gastrointestinal discomfort, and weight gain), and increased suicide risk. Benzodiazepine anxiolytics are also clinically important, but cautious use is required because of side effects such as sedation and muscle relaxation, and the development of tolerance and dependence. Thus, a novel mechanism of action based on pharmacological effects that differ from those of monoamines or γ-aminobutyric acid may open new opportunities for satisfying some or all of the unmet medical needs. Relevant new chemicals are long-awaited in psychiatric disease research and treatment.

Corticotropin-releasing factor (CRF) is a 41-amino acid neuropeptide originally isolated from ovine hypothalamus [1,2], which belongs to a mammalian CRF-related peptide family whose members include urocortin 1, urocortin 2 (also known as stresscopin-related peptide), and urocortin 3 (also known as stresscopin) [3,4]. CRF and the related urocortins are known to function as important mediators of central and peripheral stress responses through their actions at one or both of two types of G protein-coupled receptors, specifically CRF_1 and CRF_2 receptors, which are present at different anatomical localizations within the brain and peripheral tissue. Distinctively, CRF_1 receptors are highly expressed in the anterior lobe of the pituitary and widely distributed throughout neocortical, limbic, and brainstem regions of the central nervous system. Meanwhile, expression of CRF_2 receptors is limited to discrete brain regions including the raphe nuclei, lateral septum, bed nucleus of the stria terminalis, amygdala, ventromedial hypothalamus, and choroid plexus [4–7].

Compelling clinical and preclinical studies suggest that abnormally persistent central CRF neurotransmission or hypothalamic-pituitary-adrenal (HPA) axis function, presumably triggered by CRF hypersecretion and sensitized CRF receptor signal transduction, contributes significantly to the pathogenesis of stress-related disorders such as MDD and anxiety disorders. Enhanced levels of CRF in cerebrospinal fluid and increased levels of CRF-expressing neurons and CRF mRNA expression in the hypothalamic paraventricular nucleus have been identified in subpopulations of patients with MDD and certain anxiety disorders [8–11]. Accordingly, a large number of small molecule CRF_1 receptor antagonists have been developed from different pharmaceuticals as novel treatments for stress-related disorders. Their application is based on the concept that abnormally enhanced central CRF_1 receptor signaling (rather than CRF_2 receptors) contributes to the pathophysiology of these disorders [12–14]. Consequently, they are being extensively studied as agents for the treatment of MDD, anxiety disorders, IBS, post-traumatic stress disorder, and addiction. In the first clinical phase IIa study, the CRF_1 receptor antagonist, 3-[6-(dimethylamino)-4-methyl-pyrid-3-yl]-2,5-dimethyl-*N*,*N*-dipropyl-pyrazolo[2,3-*a*]pyrimidin-7-amine (R121919), has been shown to decrease depression and anxiety symptoms in MDD patients [15]; therefore, clinical expectations for CRF_1 receptor antagonism are high.

Recently, we discovered the compound, *N*-cyclopropylmethyl-7-(2,6-dimethoxy-4-methoxymethylphenyl)-2-ethyl-*N*-(tetrahydro-2*H*-pyran-4-ylmethyl)pyrazolo[1,5-*a*]pyridin-3-amine tosylate (E2508), a novel CRF_1 receptor antagonist with a pyrazolo[1,5-*a*]pyridine core (Fig. 1) [16]. The compound potently binds to the human CRF_1 receptor *in vitro* and displays appropriate drug-like characteristics. Here, we have investigated the *in vivo* pharmacological profile of E2508 using several animal models of depression and anxiety. In addition, we evaluated the effect of E2508 on spontaneous locomotor activity, muscle strength, and sexual behavior in mice and rats to predict the potential risk of side effects commonly observed in the clinical use of psychiatric drugs.

A portion of this study was previously described in abstracts presented at the annual Society for Neuroscience meeting in 2012 [17,18].

2. Materials and methods

2.1. Animals

All animals were obtained from Charles River Japan Inc. (Kanagawa, Japan). Rats and mice were housed under controlled temperature (permissible limit 20–26°C), humidity (permissible limit 40–70%), lighting (12 h light/dark cycle), and ventilation (air changes 10–15 times per hour) conditions with free access to pellet food MF (Oriental Yeast Co., Tokyo, Japan) and sterilized tap water before experiments. Unless otherwise indicated, behavioral tests

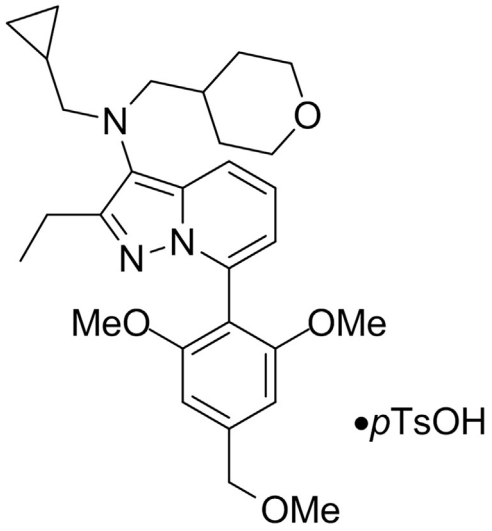

Fig. 1. Chemical structure of E2508.