European Neuropsychopharmacology (2007) **17**, 643–650

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Synergistic effects of tandospirone and selective serotonin reuptake inhibitors on the contextual conditioned fear stress response in rats

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Received 2 July 2006; received in revised form 10 January 2007; accepted 13 February 2007

KEYWORDS
Tandospirone;
5-HT_{1A} receptor agonist;
Selective serotonin reuptake inhibitor (SSRI);
Freezing;
Conditioned fear;
Anxiety

Abstract The purpose of this study was to investigate the effects of co-administration of tandospirone (a 5-HT_{1A} receptor agonist) and individual selective serotonin reuptake inhibitors (SSRIs) on the contextual conditioned fear stress, using an anxiety model in rats. One day after fear conditioning, tandospirone (0.3–3 mg/kg, s.c.), paroxetine (5–20 mg/kg, i.p.), fluvoxamine (30–60 mg/kg, i.p.) and citalopram (3–30 mg/kg, i.p.) significantly inhibited the conditioned freezing in a dose-dependent manner, whereas, 14 days after fear conditioning, the anxiolytic effects of these drugs were weakened. Fourteen days after fear conditioning, co-administration of tandospirone (0.3 mg/kg, s.c.) with each SSRI [paroxetine (5 mg/kg, i.p.), fluvoxamine (30 mg/kg, i.p.) and citalopram (10 mg/kg, i.p.)], given at subeffective doses, markedly inhibited the conditioned freezing without affecting the locomotor activities and CYP3A4-related pharmacokinetic drug–drug interaction. These results elucidate the pharmacodynamic synergistic effects of tandospirone and SSRIs. Therefore, this augmentation therapy (SSRI + 5-HT_{1A} receptor agonist) may prove useful for some anxiety disorders.

1. Introduction

Tandospirone, a serotonin-1A (5-HT_{1A}) receptor-related anxiolytic in Japan and China, is similar to buspirone and ipsapirone in the US and Europe. These 5-HT_{1A} receptor agonists have been used for the treatment of generalized anxiety disorder (GAD) (Feighner et al., 1982; Jacobson et al., 1985; Feighner and Boyer, 1989; Nishitsuji et al., 2004). In general, 5-HT_{1A} receptor agonists have a weak anxiolytic effect in comparison to benzodiazepine anxiolytics, but with the advantage of significantly low adverse effects in terms of drug dependence, abuse, and sedation (Suzuki et al., 1993; Evans et al., 1994).

Although the conventional anxiolytics (*e.g.* benzodiazepines, 5-HT_{1A} receptor agonists and tricyclic antidepressants) have been used for the treatment of anxiety disorders, recent clinical evidences have shown that selective serotonin reuptake inhibitors (SSRIs) are also effective on various anxiety disorders including GAD, panic disorder, obsessive compulsive disorder, post traumatic stress disorder, and social phobia. In addition, SSRIs are widely used as first-line drugs for the treatment of anxiety disorders (Zohar and Westenberg, 2000; Nutt, 2005), as previously established for the major depressive disorders.

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doi:10.1016/j.euroneuro.2007.02.010

Most patients have a favorable response to SSRI therapy for anxiety and mood disorders, but approximately 30% to 40% are unable to tolerate these treatments or will have an unfavorable or incomplete response (Fava and Davidson, 1996; Stocchi et al., 2003). If patients fail to respond to SSRI treatment, one treatment strategy might be to use augmentation therapy. In refractory depression, buspirone augmentations of SSRI are well documented. Co-administration of buspirone and an SSRI has been more effective than an SSRI alone in the treatment of depressive disorders (Joffe and Schuller, 1993; Dimitriou and Dimitriou, 1998; Appelberg et al., 2001). However, little is known about the efficacy of this combination (SSRI + 5-HT_{1A} receptor agonist) on anxiety disorders, although Van Ameringen et al. (1996) have shown buspirone augmentation of SSRI was effective in the treatment of social phobia. Therefore, it is important to clarify the efficacy of a 5-HT_{1A} receptor agonist augmentation of SSRIs using an anxiety animal model.

Most anxiety animal models that are now commonly used in preclinical studies, including the conflict test or elevated plus-maze test, are very useful to evaluate the efficacy of benzodiazepines. However, it is difficult to evaluate the effects of serotonergic anxiolytics such as SSRIs using these models (Borsini et al., 2002). The conditioned fear stress-induced freezing behavior can be used as a model of anxiety. Previous reports have shown that the freezing behavior is attenuated by both benzodiazepines and serotonergic anxiolytics (Hashimoto et al., 1996; Inoue et al., 1996, 2004; Li et al., 2001; Nishitsuji et al., 2006). Therefore, the conditioned fear stress model may be useful for evaluating efficacy of various therapeutic agents for anxiety disorders.

In the present study, we investigated the effects of co-administration of a 5-HT_{1A} receptor agonist (tandospirone) and the individual SSRIs (paroxetine, fluvoxamine, citalopram) using the contextual conditioned fear stress model.

2. Experimental procedures

2.1. Animals

Male Sprague-Dawley rats obtained from the Shizuoka Laboratory Animal Center (Shizuoka, Japan) weighing 250–350 g were housed in groups of four in a temperature-controlled environment (22 ± 1 °C) with access to food and water *ad libitum*. Animals were maintained on a 12 h light/dark cycle (light phase; 06:30–18:30) and tested during the light phase. All experiments were performed after a 1-week acclimatization period.

2.2. Drugs

Paroxetine hydrochloride hemihydrate (a gift from Dainippon Sumitomo Pharma, Japan) was dissolved in distilled water. Fluvoxamine maleate (a gift from Solvay Pharmaceuticals, The Netherlands), citalopram hydrobromide (a gift from Dainippon Sumitomo Pharma) and tandospirone citrate (Dainippon Sumitomo Pharma) and WAY-100635 maleate {*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide maleate} (Sigma-Aldrich, Japan) were dissolved in 0.9% sterile saline. Ketoconazole (Sigma-Aldrich) was suspended in 0.5% carboxymethyl cellulose. SSRIs and ketoconazole were injected intraperitoneally (i.p.) in a volume of 2–5 ml/kg. Tandospirone citrate and WAY-100635 maleate were injected subcutaneously (s.c.) in a volume of 1 ml/kg.

2.3. Contextual conditioned fear stress

The rats were individually subjected to inescapable electric footshock for a total of 2.5 min [five footshocks (2.5 mA scrambled shock, 30 s duration) that were delivered at intershock intervals of 35–80 s (mean 60 s)] in a shock chamber with a grid floor (19 × 22 × 20 cm; Medical Agent Co., Japan). Electric shocks were produced by a Model SGS-02D Shock Generator (Medical Agent Co.). One or 14 days after footshock, the rats were again placed in the shock chamber without footshocks and observed for 5 min. During the observation period (*i.e.* testing), the duration of freezing behavior was recorded using a time-sampling procedure (Fanselow, 1980). Every 10 s, the behavior in which the animal was currently engaged was classified as either freezing or activity. Freezing was defined as the absence of all observable movement of the skeleton and the vibrissae, except those related to respiration. All other behavior was scored as activity. The animal was classified as either freezing or active according to its behavior throughout an entire 10-sec period. The percentage score [freezing (%)] represented the number of entire 10-sec periods the animal froze for. The inhibition of freezing was also calculated [inhibition of freezing (% of vehicle)]. These procedures were approved by the Hokkaido University School of Medicine Animal Care and Use Committee, and were in compliance with the Guide for the Care and Use of Laboratory Animals, Hokkaido University School of Medicine, Japan.

2.4. Administration of tandospirone or SSRIs

One or 14 days after footshock, the rats received a single intraperitoneal injection of an SSRI [paroxetine; 5–20 mg/kg, fluvoxamine; 30–60 mg/kg; citalopram; 3–30 mg/kg] and ketoconazole (10 mg/kg) at 4 and 1.5 h before testing, respectively. Tandospirone (0.3–3 mg/kg) was subcutaneously given 30 min before testing.

2.5. Motor activity

Motor activity was measured for tandospirone (0.3 mg/kg) and SSRIs [paroxetine (5 mg/kg), fluvoxamine (30 mg/kg) and citalopram (10 mg/kg)] and their co-administration. The rats in their home cages were habituated to the testing room for 1 day. SSRIs and tandospirone were administered 4 h and 30 min before testing, respectively. In separate experiments, rats received the co-administration of an SSRI (4 h before testing) and tandospirone (30 min before testing). During the testing, rats were individually subjected to the testing cage. The motor activity was automatically recorded for 5 min by an infrared sensor that detected thermal radiation from the animals, as described by Ohmori et al. (1994). Horizontal movement was digitized and fed into a computer. Locomotion predominantly contributed to the count, but other body movements also contributed to the count when those movements contained substantial horizontal components.

2.6. Determination of tandospirone and its major metabolite 1-PP in plasma

After observing the freezing behavior, rats were immediately decapitated and blood was collected into the heparin-containing tubes. The plasma samples were prepared by centrifuging the blood samples at 1000 ×*g* for 15 min, and stored at −20 °C until analysis. The concentrations of tandospirone (free base) and 1-(2-pyrimidyl) piperazine (1-PP) in the plasma samples were determined using an LC/MS/MS system. An appropriate internal standard solution (50 μl) and distilled water (550 μl) were added to the plasma sample (100 μl). Samples were mixed thoroughly and applied on a solid-phase extraction cartridge (OASIS HLB 60 mg/3 cc, Waters Corporation,