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EFFECT OF CFMTI, AN ALLOSTERIC METABOTROPIC GLUTAMATE RECEPTOR 1 ANTAGONIST WITH ANTIPSYCHOTIC ACTIVITY, ON Fos EXPRESSION IN REGIONS OF THE BRAIN RELATED TO SCHIZOPHRENIA

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Abstract—The main purpose of this study was to explore the sites and mechanisms of action of metabotropic glutamate receptor 1 (mGluR1) blockade for antipsychotic-like activity using a Fos mapping approach, with the intent of better understanding the similarities and differences between the pharmacological actions of mGluR1 antagonists and atypical antipsychotic drugs such as clozapine. Previously, we showed that an allosteric mGluR1 antagonist (negative allosteric modulator), 2-cyclopropyl-5-[1-(2-fluoro-3-pyridinyl)-5-methyl-1H-1,2,3-triazol-4-yl]-2,3-dihydro-1H-isoindol-1-one (CFMTI), induces Fos expression in the nucleus accumbens and the medial prefrontal cortex (mPFC), but not in the dorsolateral striatum, similar to the action of clozapine. In the present study, the Fos expression profile of CFMTI was more extensively evaluated in various areas of the brain. CFMTI induced Fos expression mainly in glutamatergic neurons in the mPFC, in a manner similar to clozapine. A significant increase in Fos expression was also observed in the locous coeruleus, central amygdaloid nucleus, the bed nucleus of the stria terminalis and the primary somatosensory cortex, but not in the ventral tegmental area, dorsal raphe or lateral septum. Fos expression in orexin neurons in the lateral hypothalamic/perifornical area (LH/PFA) is known to be positively correlated with the weight gain liability of atypical antipsychotics. CFMTI did not increase Fos expression in orexin neurons in the LH/PFA, in contrast to clozapine, which does have weight gain liability. These results suggest that CFMTI has unique and shared actions on Fos expression in various regions of the brain compared with clozapine.

Key words: metabotropic glutamate receptor, negative allosteric modulator, G protein coupled receptor, c-fos, prefrontal cortex, clozapine.

Metabotropic glutamate receptor 1 (mGluR1) is one of eight G-protein-coupled receptors that are activated by L-glutamate (mGluR1–8) and is involved in the regulation of neuronal excitability, synaptic plasticity and neurotransmitter release (Conn and Pin, 1997). mGluR1 has also been implicated in the pathophysiology of CNS disorders including neuropathic pain, stroke, epilepsy, and psychiatric disorders including Parkinson's disease and anxiety (Ferraguti et al., 2008). We have recently identified a series of compounds that are potent, selective and brain-penetrable allosteric mGluR1 antagonists (negative allosteric modulators) and exhibit comparable inhibitory activity towards human and rodent mGluR1 (Suzuki et al., 2007, 2009; Satow et al., 2009). These allosteric mGluR1 antagonists show antipsychotic activity against not only dopamine-related models, but also glutamate-related models for schizophrenia (Satow et al., 2008, 2009; Satoh et al., 2009; Ito et al., 2009). These results suggest that blockade of mGluR1 could be a novel treatment for schizophrenia.

c-Fos is one of the immediate early genes, and acute and transient expression of its gene product Fos is directly and indirectly induced by diverse physiological and pharmacological stimuli (Herdegen and Leah, 1998). Fos expression has been used as a marker to map neuronal pathways and cellular activation in the CNS. One example of mapping using Fos expression has been the identification of neuroanatomical substrates and the classification of antipsychotic drugs. All clinically effective antipsychotic drugs tested induced Fos expression in the shell of the nucleus accumbens (NAcc-shell) in rodents, suggesting that the NAcc-shell is an important area for their antipsychotic action. Fos induction in the dorsolateral striatum (dlSTR) was correlated with the potency of extrapyramidal side effects (EPSs) caused by antipsychotics. Fos expression in medial prefrontal cortex (mPFC) could be associated with potency for the treatment of negative symptoms in schizophrenia (Robertson et al., 1994; Robertson and Fibiger, 1996). Therefore, profiling the Fos expression pattern could aid in the classification of novel compounds and

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Abbreviations: ARC, arcuate hypothalamic nucleus; BCIP, 5-bromo-4-chloro-3-indolyl phosphate; BNST, bed nucleus of the stria terminalis; BSA, bovine serum albumin; CeA, central amygdaloid nucleus; CFMTI, 2-cyclopropyl-5-[1-(2-fluoro-3-pyridinyl)-5-methyl-1H-1,2,3-triazol-4-yl]-2,3-dihydro-1H-isoindol-1-one; DAB, 3, 3′-diaminobenzidine; DBH, dopamin β-hydroxylase; dlPFC, dorsolateral prefrontal cortex; dlSTR, dorsolateral striatum; DMH, dorsomedial hypothalamic nucleus; DR, dorsal raphe; EDTA, ethylenediaminetetraacetic acid; EPS, extrapyramidal side effect; IHC, immunohistochemistry; LC, locous coeruleus; LH/PFA, lateral hypothalamic/perifornical area; lPFC, lateral prefrontal cortex; LS, lateral septal nucleus; mGluR1, metabotropic glutamate receptor 1; mPFC, medial prefrontal cortex; NAcc-shell, shell of the nucleus accumbens; NBT, nitro blue tetrazolium; NMDA, N-methyl-D-aspartate; oPFC, orbital PFC; PBS, phosphate-buffered saline; PCP, phencyclidine; pSSC, primary somatosensory cortex; SSC, standard saline citrate; TPBS, phosphate-buffered saline containing 0.3% Triton X-100; VGLUT1, vesicular glutamate transporter 1; VMH, ventromedial hypothalamic nucleus; VTA, ventral tegmental area.

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predict their therapeutic utility (Sumner et al., 2004). Our recent study revealed that the novel allosteric mGluR1 antagonist, 2-cyclopropyl-5-[1-(2-fluoro-3-pyridinyl)-5-methyl-1H-1,2,3-triazol-4-yl]-2,3-dihydro-1H-isoindol-1-one (CFMTI), induces Fos expression in the NAcc-shell and the mPFC, but not in the dlSTR (Satow et al., 2009), similar to that of atypical antipsychotic drugs such as clozapine and olanzapine (Robertson et al., 1994; Robertson and Fibiger, 1996). These findings were consistent with the observation that CFMTI shows significant antipsychotic-like activity without EPSs in the animal models studied (Satow et al., 2009). However, the sites and mechanisms of action of the antipsychotic-like activity mediated by mGluR1 blockade still remain to be clarified.

The main purpose of the present study was to explore the sites and mechanisms of action of mGluR1 blockade for antipsychotic-like activity using a Fos mapping approach. These studies were designed to provide a better understanding of the similarities and differences between the pharmacological actions of mGluR1 antagonists and atypical antipsychotic drugs such as clozapine. Thus, the effects of CFMTI on Fos expression in various regions of the brain were evaluated. In particular, we investigated the activity of CFMTI on Fos expression in regions of the brain related with negative symptoms, cognitive functions, and weight gain liability, which are current issues in treatments for schizophrenia (Allison and Casey, 2001; Nasrallah, 2003; Conn et al., 2008). In addition, the therapeutic utility of the allosteric mGluR1 antagonist for the treatment of schizophrenia, based on the results of Fos mapping, is discussed.

EXPERIMENTAL PROCEDURES

Animals

All experiments were performed using adult male Sprague–Dawley rats (Charles River Laboratories Japan, Kanagawa, Japan). Animals were housed in an air-conditioned room with a 12-h light/dark cycle (lights off at 7:00 PM) and allowed *ad libitum* access to food (CE-2; Clea Japan, Inc., Tokyo, Japan) and tap water. At least 6 days were allowed for acclimatization to the facility before starting experiments. Drug administration was performed between 10:00 AM and 12:00 PM. The vehicle controls were handled the same way at the same time. All experiments were approved by our Institutional Animal Care and Use Committee, minimizing the number of animals used and their suffering.

Compounds

CFMTI (Satow et al., 2009) was synthesized in-house, and used in all experiments as the free base suspended in 0.5% methylcellulose for oral administration. Clozapine (Tocris Bioscience, Ellisville, MO, USA) was dissolved in HCl and then diluted with physiological saline for i.p. administration. Haloperidol (Serenece; Dainippon Sumitomo Pharma, Osaka, Japan) was diluted with distilled water for s.c. administration. The dosing volume was 1 mL/kg.

Fos immunohistochemistry (IHC)

Rats were administered CFMTI (10–30 mg/kg p.o.), clozapine (30 mg/kg i.p.) or haloperidol (1 mg/kg s.c.) and then returned to their cages. The rats were decapitated 2 h after injection of the test drug or the corresponding vehicle.

The brains were rapidly removed from the skulls and immediately frozen in dry ice-cooled 2-methylbutane (−40 °C) and then stored at −80 °C until sectioning. The 20-μm thick coronal frozen sections were cut using a cryostat microtome (Microm HM 500 O; Microm, Walldorf, Germany) and thaw-mounted on SuperFrost Plus microscope slides (Thermo Fisher Scientific, Waltham, MA, USA). Coronal sections were taken at seven levels, corresponding approximately to coordinates AP +2.7 for the mPFC and the lateral prefrontal cortex (lPFC), +1.6 for the NAcc-shell, the dlSTR and the primary somatosensory cortex (pSSC), −0.2 for the bed nucleus of the stria terminalis (BNST) and the lateral septal nucleus (LS), −2.8 for the central amygdaloid nucleus (CeA), the lateral hypothalamic/perifornical area (LH/PFA), the dorsomedial hypothalamic nucleus (DMH), the ventromedial hypothalamic nucleus (VMH) and the arcuate hypothalamic nucleus (ARC), −5.8 for the ventral tegmental area (VTA), −7.8 for the dorsal raphe (DR) and −9.6 mm for the locous coeruleus (LC), from bregma according to the atlas of Paxinos and Watson (Paxinos and Watson, 1998). The sections were air-dried at room temperature and stored at −80 °C until use.

Slides were brought to room temperature and immersed in 4% formaldehyde in phosphate-buffered saline (PBS, pH 7.4) for 30 min and then washed three times 5 min each (3×5 min) in PBS. The slides were placed in PBS containing 0.3% hydrogen peroxide for 30 min to quench endogenous peroxidase and washed 3×5 min in phosphate-buffered saline containing 0.3% Trition X-100 (TPBS) with 1% (w/v) bovine serum albumin (BSA) Fraction V (Sigma-Aldrich, St. Louis, MO, USA). The sections were incubated in rabbit anti-c-Fos serum (1:20,000, Ab-5, Calbiochem, San Diego, CA, USA) in TPBS with 1% BSA overnight at 4 °C, followed by washing (3×5 min) in TPBS with 1% BSA. Antigenic sites were visualized using a Vectastain Elite ABC kit (rabbit IgG) and DAB Substrate Kit for peroxidase (Vector Laboratories, Burlingame, CA, USA). In brief, sections were incubated in biotinylated goat anti-rabbit IgG (1:1000) in TPBS with 1% BSA for 1 h at room temperature, followed by washing (3×5 min) in TPBS with 1% BSA. Sections were consecutively reacted with avidin–biotin peroxidase complex for 1 h and then washed (3×5 min) in TPBS. The Fos immunoreactive nuclei were visualized with nickel-intensified DAB, followed by washing (3×5 min) in ice-cold PBS. The sections were then dehydrated using a series of graded ethanol, cleared in xylene, and coverslipped (Entellan New; Merck, Darmstadt, Germany). Automated computer software (ImagePro Plus, Media Cybernetics, Inc., Bethesda, MD, USA) was used to count Fos-like immunoreactivities in the brain sections, blindly to the treatment. In the prefrontal cortex, the number of Fos-positive cells was counted in the prelimbic area of the mPFC and the agranular insular area in the lPFC.

Dual labeling for Fos protein with neuron markers using IHC

In dual labeling, visualization of Fos protein was followed by the detection of dopamine β-hydroxylase (DBH) or orexin. Immunohistochemistry of Fos protein was performed as described above unless stated otherwise. Swine biotinylated anti-rabbit Ig (1:300, E 0353, DakoCytomation, Glostrup, Denmark) was used as a secondary antibody for the detection of Fos protein. After visualization of the Fos immunoreactive nuclei with nickel-intensified DAB, the sections were incubated in PBS containing 1% hydrogen peroxide and 0.1% sodium azide for 30 min and then washed (3×5 min) in TPBS with 1% BSA. Primary antibody reaction and subsequent procedures were performed as described above unless stated otherwise. For the detection of DBH protein, mouse anti-DBH monoclonal antibody (1:10000, MAB308, Chemicon, Temecula, CA, USA) and rabbit biotinylated anti-mouse Ig antibody (1:500, E 0464, DakoCytomation) were used as the primary and secondary antibodies, respectively. For the detection of orexin peptide, goat anti-orexin A antibody (sc-8070, Santa Cruz Biotechnology,