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Antidepressant/anxiolytic potential and adverse effect liabilities of melanin-concentrating hormone receptor 1 antagonists in animal models

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ABSTRACT

Melanin-concentrating hormone receptor 1 (MCH1 receptor) is known to be involved in the control of mood and stress, in addition to the regulation of feeding. Here, we report further evidence that the blockade of the MCH1 receptor exhibits antidepressant and anxiolytic-like effects in a variety of animal models using TASP0382650 and TASP0489838, newly synthesized MCH1 receptor antagonists, with different scaffolds. Both TASP0382650 and TASP0489838 exhibited high affinities for human MCH1 receptor with IC_{50} values of 7.13 and 3.80 nM, respectively. Both compounds showed potent antagonist activities at the MCH1 receptor, as assessed using MCH-increased [^{35}S]GTPγS binding to human MCH1 receptor and an MCH-induced $[Ca^{2+}]_i$ assay in rat MCH1 receptor expressing cells. In contrast, neither TASP0382650 nor TASP0489838 showed an affinity for the MCH2 receptor, another MCH receptor subtype. The oral administration of TASP0382650 or TASP0489838 significantly reduced the immobility time during the forced swimming test in rats, and reduced hyperemotionality induced by an olfactory bulbectomy, both of which are indicative of an antidepressant-like potential. In the olfactory bulbectomy model, the antidepressant effect of TASP0382650 appeared following a single administration, suggesting a faster onset of action, compared with current medications. Moreover, both TASP0382650 and TASP0489838 exhibited anxiolytic effects in several animal models of anxiety. In contrast, both TASP0382650 and TASP0489838 did not affect spontaneous locomotor activity, motor function, spatial memory during the Morris water maze task, or the convulsion threshold to pentylenetetrazole. These findings provide additional evidence that the blockade of the MCH1 receptor exhibits antidepressant- and anxiolytic activities with no adverse effects in experimental animal models.

1. Introduction

Depression and anxiety disorders are among the most prevalent forms of mental illness. Current medications for both depression and anxiety disorders are dominated by drugs that act on monoaminergic transmissions or GABAergic transmission. Although currently prescribed antidepressants have been proven to be useful for the treatment of both depression and anxiety disorders, they are not ideal because of their slow onset of action, low response rate and adverse effects that often result in the discontinuation of medication in many patients. Likewise, the long-time use of benzodiazepine anxiolytics increases the risk of drug dependence and tolerance. Therefore, an urgent need exists for novel drugs that are more effective and have fewer adverse effects for the treatment of both depression and anxiety disorders.

Several neuropeptides in the brain that have roles in the regulation of stress responses and emotion are thought to be involved in the pathophysiology of depression and anxiety disorders (Holmes et al., 2003; Griebel and Holsboer, 2012). Indeed, agents acting on neuropeptide receptors such as corticotropin-releasing factor 1 (CRF1) receptor, neurokinin 1 receptor and vasopressin 1b (V1b) receptor have been reported to exert antidepressant and anxiolytic effects in several animal models (Griebel and Holsboer, 2012), although the outcomes of clinical trials have not necessarily been encouraging (Binneman et al., 2008; Griebel et al., 2012; Keller et al., 2006). Among these neuropeptides, melanin-concentrating hormone (MCH), which is produced mainly in the lateral hypothalamus and zona incerta (Bittencourt et al., 1992), is reportedly involved in a wide range of physiological processes including feeding, stress responses, and neuroendocrine functions, and its receptor, namely, the MCH1 receptor, may mediate these physiological

Abbreviations: AVOV, analysis of variance; CNS, central nervous system; HPA, hypothalamus–pituitary axis; MCH, melanin-concentrating hormone; MCH1 receptor, melanin-concentrating hormone receptor 1; TASP0382650, N-(cis-4-{[6-(dimethylamino)-2-methylpyrimidin-4-yl]amino}cyclohexyl)-3,4,5-trifluorobenzamide; TASP0489838, 3-methoxy-N-[1-({7-[(2-methoxyethoxy)methyl]naphthalen-2-yl}methyl)piperidin-4-yl]benzamide.

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functions. Several lines of evidence suggest that MCH and the MCH1 receptor are involved in the pathophysiology of depression and anxiety. Indeed, the injection of MCH reportedly induces behavioral phenotypes that resemble depression and anxiety (Georgescu et al., 2005; Smith et al., 2006) and stress-related neurochemical alterations that are typically observed in depressed patients (Smith et al., 2006).

In contrast, several groups, including our group, have reported that MCH1 receptor antagonists exert antidepressant and anxiolytic effects in several animal models (Borowsky et al., 2002; Chaki et al., 2005; Gehlert et al., 2009; Smith et al., 2006), which are consistent with some of the behavioral phenotypes of knockout mice lacking the MCH1 receptor (Roy et al., 2006, 2007; Smith et al., 2006). However, to further predict the efficacy of MCH1 receptor antagonists and the possible advantages over currently prescribed antidepressants/anxiolytics, thorough pharmacological evaluations are necessary in a variety of animal models to predict both the antidepressant/anxiolytic potential and possible adverse effect liabilities using potent and selective MCH1 receptor antagonists with distinct scaffolds.

Recently, we synthesized two novel non-peptide and potent MCH1 receptor antagonists, N-(cis-4-{[6-(dimethylamino)-2-methylpyrimidin-4-yl]amino}cyclohexyl)-3,4,5-trifluorobenzamide (TASP0382650) and 3-methoxy-N-[1-({7-[(2-methoxyethoxy)methyl]naphthalen-2-yl}methyl)piperidin-4-yl]benzamide (TASP0489838), both of which were derived from different scaffolds (Fig. 1). In the present study, we first evaluated the antidepressant and anxiolytic potentials of these compounds using several animal models. We then elucidated the advantages of MCH1 receptor antagonists over currently prescribed antidepressants and anxiolytics. Finally, we evaluated the adverse effect liabilities of MCH1 receptor antagonists.

2. Materials and methods

2.1. Animals

Male ICR mice (Charles River, Yokohama, Japan) were used for stress-induced hyperthermia, locomotor activity, rotarod test, hexobarbital-induced sleeping and pentylenetetrazole (PTZ)-induced convulsion. Male Sprague–Dawley (SD) rats (Charles River, Yokohama, Japan) were used for the forced swimming test, the conditioned fear stress paradigm, the social interaction test, the elevated plus-maze test, and evaluations of locomotor activity, rotarod test, hexobarbital-induced sleeping, catalepsy, and flat body posture. Male Wistar rats (Charles River, Yokohama, Japan) were used for the olfactory bulbectomy model and the Morris water maze test. Guinea pig pups (5 days-old) were obtained from SLC (Hamamatsu, Japan). All animals were maintained under a 12 h light/dark cycle (light on at 7:00 AM) in a temperature-and humidity-controlled holding room. Food and water were available *ad libitum*.

2.2. Ethics

All the studies were reviewed by the Taisho Pharmaceutical Co., Ltd. Animal Care Committee and met the Japanese Experimental Animal Research Association standards, as defined in the *Guidelines for Animal Experiments* (1987).

2.3. Chemicals

TASP0382650 hydrochloride or methanesulfonate and TASP0489838 hydrobromide were synthesized at Taisho Medicinal Research Laboratories. Except for the determination of affinities for the 5-HT_{1A} receptor and the 5-HT_{2B} receptor, in which TASP0382650 hydrochloride was used, TASP0382650 methanesulfonate was used for all the studies. [^{125}I][Phe^{13},Tyr^{19}]MCH (specific radioactivity: 74 TBq/mmol) and [^{3}H]8-OH-DPAT (specific radioactivity: 7.99 TBq/mmol) were purchased from Amersham Biosciences UK, Ltd (Buckinghamshire, UK). [^{125}I]Lysergic acid diethylamide (LSD) (specific radioactivity: 81.4 TBq/mmol) and [^{35}S]GTPγS (specific radioactivity: 46.25 TBq/mmol) were purchased from PerkinElmer Life Sciences Inc. (Boston, MA, USA). MCH was purchased from Sigma-Aldrich (St. Louis, MO, USA). Human MCH1 receptor-expressing Chinese hamster ovary (CHO) cell membranes and human 5-HT_{2B} receptor-expressing CHO cell membranes were purchased from Euroscreen (Brussels, Belgium). Human 5-HT_{1A} receptor-expressing CHO cell membranes were purchased from PerkinElmer Life Sciences Inc. (Boston, MA, USA). TASP0382650 and TASP0489838 were dissolved in 10% 2-hydroxypropyl-β-cyclodextrin for the in vivo studies, and in dimethylsulfoxide for the in vitro studies.

2.4. [^{125}I][Phe^{13},Tyr^{19}]MCH binding

The membranes of CHO-K1 cells expressing the human MCH1 receptor were suspended in 25 mM HEPES buffer (pH 7.4) containing 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.5 mM phenylmethylsulfonyl fluoride and 0.2% bovine serum albumin at a protein concentration of 12.5 μg/mL. Membranes were incubated with [^{125}I][Phe^{13},Tyr^{19}]MCH (0.1 nM) for 2 h at 25 °C. The reaction was terminated by rapid filtration under vacuum through a UniFilter GF/C microplate (PerkinElmer Life Sciences, Boston, MA, USA) presoaked with 0.3% polyethyleneimine, after which the filters were washed three times with 0.3 mL of phosphate buffered saline containing 0.5 M NaCl using a UniFliter96 harvester (Packard Instruments, Meriden, CT, USA). The filter-bound activity was counted in a TopCount NXT Microplate Scintillation and Luminescence Counter C384V01J (Packard Instruments, Meriden, CT, USA).

2.5. [^{35}S]GTPγS binding

The membranes of CHO cells expressing human MCH1 receptor were suspended in assay buffer (20 mM HEPES buffer containing 100 mM NaCl, 10 mM $MgCl_2$, 1 μM GDP, 10 μg/mL saponin and 0.2% bovine serum albumin [pH 7.4]) to yield a protein concentration of 2 μg/assay (TASP0382650) or 4 μg/assay (TASP0489838). The membranes were pre-incubated with various concentrations of TASP0382650 and 4 nM MCH for 20 min at 30 °C or with various concentrations of TASP0489838 and 5 nM MCH for 15 min at 25 °C. [^{35}S]GTPγS (0.1 nM) was then added, and the membranes were incubated for 30 min at 30 °C (TASP0382650) or 25 °C (TASP0489838). For the agonist activity experiments, 2 μM of GDP, instead of 1 μM of GDP, was used, and the assay was performed at a protein concentration of 4 μg/assay. The reaction was terminated by rapid filtration under a vacuum through a UniFilter GF/C microplate (PerkinElmer Life Sciences, Boston, MA, USA) presoaked with assay buffer, after which the filters were washed three

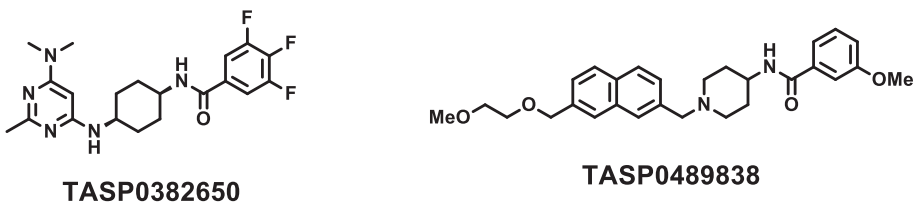

Fig. 1. Chemical structures of TASP0382650 and TASP0489838.