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Anxiolytic-like effect of a selective and non-peptidergic melanocortin 4 receptor antagonist, MCL0129, in a social interaction test

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Abstract

The social interaction test is an animal behavioral test of anxiety. Brain melanocortins such as α-melanocyte stimulating hormone (α-MSH) and adrenocorticotropic hormone (ACTH) have anxiogenic effects in this test. Melanocortins have five receptor subtypes (MC1–MC5). Among them, MC3 and MC4 receptor are mainly expressed in the brain. We investigated the involvement of MC4 receptor in a social interaction test, using Ac-[Nle^4,Asp^5,D-Phe^7,Lys^{10}]alpha-MSH-(4-10)-NH2 (MT II), an MC4 receptor agonist, and 1-[(S)-2-(4-fluorophenyl)-2-(4-isopropylpiperadin-1-yl)ethyl]-4-[4-(2-methoxynaphthalen-1-yl)butyl]piperazine (MCL0129), a selective and nonpeptide MC4 receptor antagonist. MT II dose-dependently and significantly reduced the time spent in social interaction. Acute administration of MCL0129 had no effect on the results of this test. In contrast, when given repeatedly for 1 week, MCL0129 significantly and dose-dependently increased the time spent in social interaction without affecting locomotor activity. These results suggest that MC4 receptor is involved in social interaction, and that MCL0129, an MC4 receptor antagonist, has an anxiolytic-like effect in this model.

Keywords: MCL0129; Melanocortin; Anxiolytic; Social interaction model; Rat

1. Introduction

Many papers have argued that brain melanocortins (MCs), derived from pro-opiomelanocortin (POMC) by enzymatic processing, are involved in various physiological events, including those of learning and memory (De Wied and Jolles, 1982), thermoregulation (Murphy et al., 1983), analgesia (Vrinten et al., 2000, 2001), stress response (Dunn et al., 1979; De Barioglio et al., 1991; Adan et al., 1999; Von Frijtag et al., 1998) and feeding behavior (Poggioli et al., 1986).

Among MCs, it has been reported that α-melanocyte stimulating hormone (α-MSH) and adrenocorticotropic hormone (ACTH) induce excessive grooming behavior in rats (De Barioglio et al., 1991; Adan et al., 1999), while the antiserum to ACTH reduces novelty-induced grooming (Dunn et al., 1979). Injection of ACTH activates the hypothalamus-pituitary-adrenal (HPA) axis (Von Frijtag et al., 1998). Abnormal HPA activity has been implicated in a variety of stress-related conditions, including HPA over-activation in depression and anxiety. Moreover, α-MSH and ACTH have been shown to exert anxiogenic-like effects in various behavioral tests such as Vogel conflict (Corda et al., 1990), aggressive behavior (Gonzalez et al., 1996), isolation-induced vocalization (Panksepp and Normansell, 1990) and social interaction (File and Clarke, 1980).

The social interaction test of anxiety has been validated physiologically, behaviorally and pharmacologically (File and Seth, 2003). There are four test conditions: low light/familiar arena (generating the lowest level of anxiety); high light/familiar arena and low light/unfamiliar arena (generating moderate levels of anxiety); and high light/unfamiliar arena (generating the highest level of anxiety). Following social interaction, rat plasma concentrations of ACTH are significantly higher after testing in the high light condition

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than after testing in the low light condition (File, 1984). This indicates that exposure to social interaction changes stress hormones and generates behavioral changes indicative of anxiety. Moreover, intracerebroventricular administration of α-MSH and ACTH reduced the time of social interaction (File and Clarke, 1980). These findings indicate that stress and brain melanocortins such as α-MSH and ACTH enhance anxiogenic-like behavior in the social interaction test.

To date, five receptor subtypes (MC1–MC5) have been cloned for MC (Chhallani and Wikberg, 1992; Chhallani et al., 1993; Mountjoy et al., 1992; Gantz et al., 1993a,b). Of these, MC3 and MC4 mRNA are the most commonly expressed in the brain (Gantz et al., 1993a,b). Several lines of evidence indicate that the MC4 receptor is related to stress-induced behavior. MC4 receptor agonists induce grooming behavior in rats, while MC4 receptor antagonist, SHU9119, reduces this behavior (Adan et al., 1999). Moreover, another selective MC4 receptor antagonist, HS014, blocks immobilization stress-induced anorexia in rats (Vergoni et al., 1999). MC4 receptor may thus play an important role in stress responses involving α-MSH and ACTH.

MCL0129, 1-[(S)-2-(4-fluorophenyl)-2-(4-isopropylpiperadin-1-yl)ethyl]-4-[4-(2-methoxynaphthalen-1-yl)butyl]-piperazine, is a nonpeptide-selective antagonist for the MC4 receptor. Based on our previous experiments, we found that MCL0129 showed a high affinity for MC4 receptor (IC50=8 nM), while it did not show affinities for other receptors (Chaki et al., 2003).

This report describes potential links between MC4 receptor and social interaction, based on the social interaction test performed using Ac-[Nle^4,Asp^5,D-Phe^7,Lys^{10}]alpha-MSH-(4-10)-NH2 (MT II), an MC4 receptor agonist, and MCL0129 as pharmacological tools.

2. Material and methods

2.1. Animals

Male Sprague–Dawley rats (purchased from Charles River), weighing approximately 300 g, were housed individually for 7 days before the social interaction test. Animals were maintained in a temperature- and humidity-controlled holding room (lights on from 0700 to 1900 h). Food and water were available ad libitum. All studies were reviewed by the Taisho Pharmaceutical Co., Ltd. Animal Care Committee and met the Japanese Experimental Animal Research Association standards, as specified in *Guidelines for Animal Experiments* (1987).

2.2. Surgery

In experiments for intracerebroventricular infusion, rats were surgically equipped with a single cannula placed above the lateral ventricle. Animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p.), and placed in a stereotaxic apparatus (Narishige, Tokyo, Japan) where a 7-mm long, 23-gauge stainless steel guide cannula was placed to within 1 mm of the ventricle and anchored to the skull with screws and dental cement. The implantation coordinates were 1.0 mm posterior to the bregma, 1.2 mm lateral to the midline, and 4.5 mm ventral to the cortical surface according to the rat brain atlas of Paxinos and Watson. The rats were allowed to recover for 1 week, and then adapted to the injection procedure. At the end of the study period, rats were killed by decapitation after injection of blue ink. Brains were immediately examined for presence of dye in the ventricles. Only those rats with correct cannula placement were included in the data analysis.

2.3. Drugs

Ac-[Nle^4,Asp^5,D-Phe^7,Lys^{10}]alpha-MSH-(4-10)-NH2 (MT II) (purchased from Funakoshi, Tokyo, Japan) was dissolved in buffered artificial cerebrospinal fluid (ACSF) containing 0.1% bovine serum albumin. 1-[(S)-2-(4-fluorophenyl)-2-(4-isopropylpiperadin-1-yl)ethyl]-4-[4-(2-methoxynaphthalen-1-yl)butyl]piperazine, MCL0129 (synthesized at Taisho Pharmaceutical Medicinal Research Laboratories) was dissolved in 0.3% Tween 80/saline solution. Chroldiazepoxide (CDP) (purchased from Sigma, Tokyo, Japan) was suspended in 0.4% carboxymethylcellulose (CMC). MTII is a peptidomimetic MC4 receptor agonist, and brain penetration is expected to be very low. Therefore, MT II was injected intracerebroventricularly (10 μl/rat) through the cannula at 10 μl/min 30 min prior to the test. MCL0129 and CDP were administered orally 1 h prior to the tests in 2 ml/kg body weight. Our pharmacokinetic study showed that MCL0129 showed excellent brain penetration when administered orally, and it has been reported that oral administration of MCL0129 is effective in animal models (Chaki et al., 2003). For sub-chronic treatment, rats were given drugs three times in succession at an interval of 2 days. The experiments were carried out 1 h after the last administration.

2.4. Social interaction test

Sensitive to the effects of anxiolytic drugs (File and Hyde, 1978), this test is performed in an open-field apparatus placed in an isolated chamber. The apparatus was an open-topped perspex box (45×27×30 cm) with a solid floor. Tests were conducted in an illuminated room (300 radiometric lx) using a method based on the model presented by File (1980). The base of the arena was marked by fifteen 9×9 cm squares. A camera was mounted above the arena. The rats were allocated to test pairs on the basis of weight. Both rats of a pair always received the same drug treatment. For the 2 days before the test began, the rats were allowed to explore the apparatus individually for 4 min per

day. The rats were thus able to familiarize themselves with the apparatus but not with their partner. In the test, rats were placed in the test arena for a 10-min trial. The mean time spent in social interaction (defined as sniffing, following, social grooming and crawling under or over) for the pair was scored. The number of line crossings made by each rat was also counted as a measure of general motor activity.

2.5. Statistical analysis

The results were analyzed by Dunnett's test followed by one-way ANOVA.

3. Results

3.1. Social interaction

3.1.1. Acute treatment with MT II

Intracerebroventricular administration of MT II reduced in the time spent in social interaction, significantly and dose-dependently [$F(2,21)=32.00$, $p<0.001$] (Fig. 1a).

3.1.2. Acute treatment with CDP and MCL0129

For testing 1 h after a single dose of the positive control, CDP, a significant anxiolytic effect was observed in the social interaction test, as revealed by one-way ANOVA [$F(4,35)=4.03$, $p<0.01$]. CDP caused a bell-shaped increase in social interaction (Fig. 2a). The increase in social interaction by CDP was significant at doses of 4 mg/kg ($p<0.05$) and 8 mg/kg ($p<0.01$). A single dose of MCL0129 failed to show any significant effect on social interaction at any the doses tested [$F(3,28)=1.52$] (Fig. 2b).

3.1.3. Sub-chronic treatment with MCL0129

For testing 1 h after the last administration of MCL0129 and vehicle, there was a significant drug effect on social interaction as revealed by one-way ANOVA [$F(3,28)=8.00$, $p<0.001$] (Fig. 2c). Social interaction time in the MCL0129 3 and 10 mg/kg-treated groups was significantly longer than in the vehicle-treated group ($p<0.01$, and $p<0.001$, respectively).

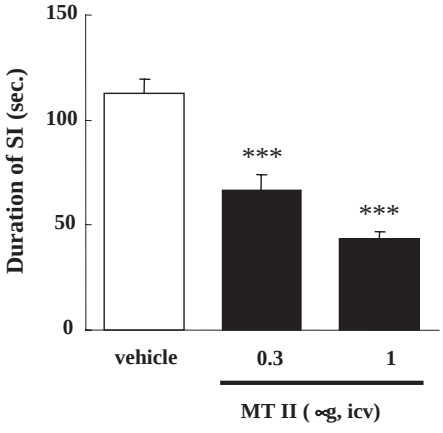

Fig. 1. Mean time spent in social interaction with i.c.v. treatment of MT II. Data represent the mean±S.E.M. ($n=8$). ***$p<0.001$ versus vehicle-treated group (Dunnett's test).

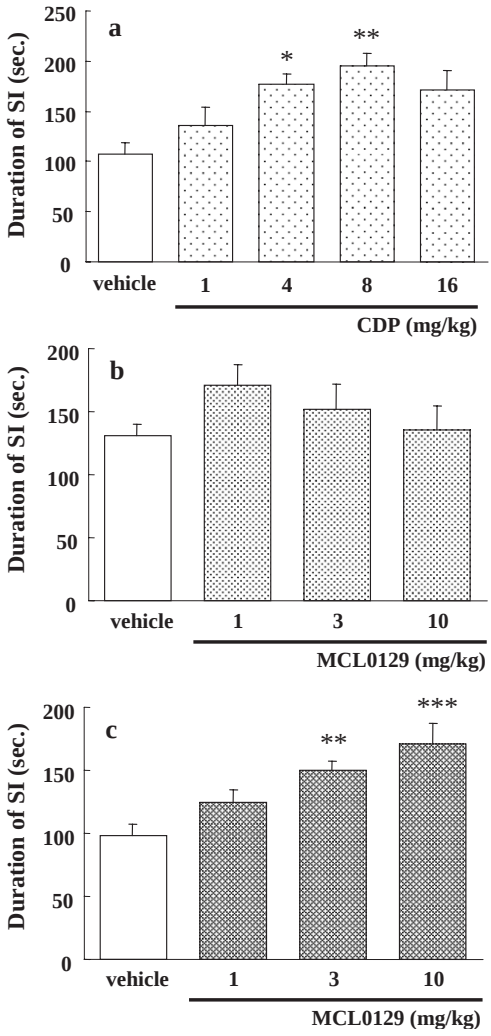

Fig. 2. Mean time spent in social interaction. (a) Chlordiazeoxide (CDP), (b) single treatment with MCL0129, (c) sub-chronic treatment (rats were administered the drug three times at 2-day interval in the course of 1 week) with MCL0129. Data represent the mean±S.E.M. ($n=8$). *$p<0.05$, **$p<0.01$, ***$p<0.001$ versus vehicle-treated group (Dunnett's test).

3.1.4. Locomotion

No significant effect on line crossing was observed in the social interaction test with either acute or chronic MCL0129 treatment, indicating that increase in social behavior with MCL0129 was not ascribed to alteration in locomotor activity (Fig. 3a,b).

4. Discussion

In the present study, we found that MC4 receptor agonist induced an anxiogenic-like activity in the social interaction test, while CDP used as a positive control, significantly

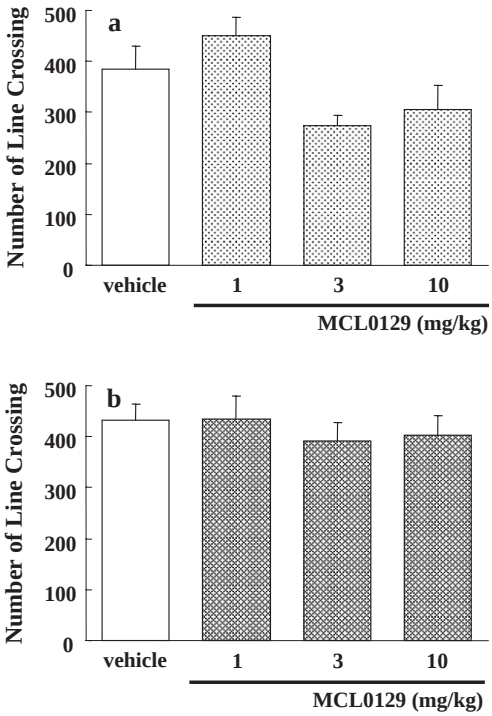

Fig. 3. Number of line crossings in social interaction test. (a) Single treatment with MCL0129, (b) sub-chronic treatment with MCL0129. Data represent the mean±S.E.M. (n=8). No significant difference was observed (Dunnett's test).

increased social interaction time in the present condition. In contrast, when administered repeatedly for 1 week, MCL0129 had no effect on locomotion and caused an anxiolytic-like increase in social interaction, with significant effects observed after administration of the 3 and 10 mg/kg doses. A single administration of MCL0129 did not produce significant anxiolytic-like activity in the social interaction test. In the previous study, we reported that MCL0129 is a selective MC4 receptor antagonist (Chaki et al., 2003), indicating that the effect of MCL0129 is mediated through the blockade of the MC4 receptor. This is the first report in which MC4 receptor has been linked to anxiety-like results in the social interaction test.

In the present study, intracerebroventricular injection of MT II, an MC4 agonist, significantly reduced the time of social interaction, suggesting that stimulation of MC4 receptor causes anxiogenic-like effect. This result is consistent with the previous report that α-MSH and ACTH, endogenous MC receptor agonists, exhibited anxiogenic-like effect in the social interaction test (File and Clarke, 1980).

Both MC3 receptor and MC4 receptor are expressed in the brain (Gantz et al., 1993a,b), raising the possibility of the involvement of MC3 receptor in social interaction. It has been reported that MC3 receptor may play a negligible role in stress response. Grooming behavior has been observed in situations inducing mild stress, such as exposure to unfamiliar situations. The selective MC3 agonist, Nle-γ-MSH, has been reported not to induce grooming behavior, whereas MC4 antagonists block α-MSH or novelty-induced excessive grooming behavior (Adan et al., 1999). Moreover, it has been reported that stimulation of MC4 receptor activates the HPA axis, while MC3 receptor may not be involved in the regulation of HPA axis activation (Von Frijtag et al., 1998). Thus, MC3 receptor may be less involved in stress response than MC4 receptor. It has been reported that stress markedly affects social interaction, and that various kinds of stress cause anxiogenic effects in this paradigm (File, 1994; File and Pellow, 1985; Zangrossi and File, 1992). In this respect, MC4 receptor likely plays a greater role in social interaction than MC3 receptor.

In the social interaction test, the septum has been identified as one of the primary brain regions important for mediating the effects of ACTH (Clarke and File, 1983). It has been reported that the lateral septum plays a role in mediating anxiolytic functions (Thomas, 1988). Electrical stimulation of the lateral septum has fear-relieving properties, and anxiolytic-like effects, while lesions of the lateral septum result in increased fear and anxiogenic changes (Yadin et al., 1993). A recent study found that the lateral septum displayed a high density of expression of MC4 receptor mRNA (Kishi et al., 2003). Moreover, intracerebroventricular administration of Agouti-related peptide, an endogenous MC3 and MC4 receptor antagonist, evoked c-Fos-like immunoreactivity in the lateral septum (Hagan et al., 2001). Similar increases in c-Fos-like immunoreactivity in the lateral septum have been observed following ventricular injection of NDP-α-MSH, an MC receptor agonist (Brown et al., 1998). Therefore, it is likely that the blockade of MC4 receptor in the lateral septum plays an important role in social interaction. Because we cannot exclude the possibility that MC4 receptor in other brain regions is involved in social interaction, more definitive experiments are needed.

Only repeated administration of MCL0129 caused an anxiolytic-like increase in social interaction. These anxiolytic-like effects of MCL0129 resembled those of paroxetine, a selective serotonin reuptake inhibitor (SSRI) (Lightowler et al., 1994). SSRIs are well-established as antidepressants in animal models and in clinical studies. In studies involving the rat forced swimming test, it has been reported that SSRIs increase swimming behavior, while drugs interfering with noradrenergic transmission increase climbing behavior (Detke et al., 1995). The marble burying behavior test in mice has been suggested to be a useful model for evaluating anti-OCD drugs. SSRIs inhibited this burying behavior in mice (Njung'e and Handley, 1991a,b). Previous studies in our laboratory have shown that MCL0129 has antidepressant-like effects in the forced swimming test (Chaki et al., 2003). In this test, MCL0129, similar to SSRIs, increased swimming behavior without affecting climbing behavior. Moreover, MCL0129 was effective in inhibiting marble-burying behavior (Chaki et al., 2003). These results suggest that behavioral responses

in animal models to MCL0129 administration mimic those of SSRIs. Interactions between MC4 receptor and serotonergic transmission have been suggested. The dorsal raphe nucleus (DRN) is one of the brain regions in which both MC4 receptor mRNA and POMC-immunoreactive axons are localized (Kishi et al., 2003; Zheng et al., 1991). Recently, we reported that an MC4 receptor agonist alters the neuronal activity of DRN serotonergic neurons (Kawashima et al., 2003). This interaction involving MC4 receptor and serotonergic neurons may partially explain the anxiolytic/anxiogenic effects of MC4 antagonist/agonist in the social interaction test.

The reason for the lack of anxiolytic-like effects in the social interaction test following acute administration of MCL0129 remains unknown. MCL0129 penetrates the brain relatively slowly (unpublished data). In this test, the single 1 h dosing may not have been sufficient to achieve MCL0129 brain concentrations exerting anxiolytic-like effects. It is also conceivable that subsequent alterations in neural circuits following the blockade of MC4 receptor may be required to produce anxiolytic effects in the social interaction test.

In conclusion, the present data clearly indicate that MC4 receptor is involved in social interaction. The data also indicates that a sub-chronic blockade of MC4 receptor produces anxiolytic effects.

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