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Anxiolytic Effects of the MCH1R Antagonist TPI 1361-17

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Abstract Melanin-concentrating hormone (MCH) is a hypothalamic neuropeptide that acts on the MCH1 receptor. MCH1R is expressed widely throughout the brain, particularly in regions thought to be involved in the regulation of stress and emotional response. The role of MCH in anxiety has been controversial, however. Central administration of MCH has been reported to promote or reduce anxiety-like behaviors. The anxiolytic activity of several MCH1R antagonists has also been debated. To address this issue, we have tested whether TPI 1361-17, a highly specific and high affinity MCH1R antagonist, exerts anxiolytic effects in two commonly used models of anxiety, the elevated plus maze and the light–dark transition test. We show that this MCH1R antagonist exerts potent anxiolytic effects in both assays. Our study therefore supports previous studies indicating that MCH1R antagonists may be useful in the treatment of anxiety.

Keywords Melanin-concentrating hormone · Anxiety · G protein-coupled receptor · Neuropeptide

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Introduction

Melanin-concentrating hormone (MCH) was originally isolated from salmon pituitaries as a hormone that induces paling of the skin in teleost fish (Kawaguchi et al. 1983). Subsequently, MCH was identified in the mammalian hypothalamus as a cyclic nonadecapeptide (Vaughan et al. 1989). In mammals, MCH is expressed predominantly in neurons of the lateral hypothalamus and the zona incerta which project broadly throughout the brain to regions involved in the regulation of feeding, energy homeostasis, and mood related behaviors (Bittencourt et al. 1992). MCH exerts its physiological and behavioral effects through two G protein-coupled receptors, MCH1R (Bachner et al. 1999; Chambers et al. 1999; Lembo et al. 1999; Saito et al. 1999; Shimomura et al. 1999) and MCH2R (Sailer et al. 2001), but only the MCH1 receptor is expressed in rodents (Tan et al. 2002). MCH1 receptors are widely distributed throughout the CNS with particularly dense expression in the cortex, hippocampus, amygdala, locus coeruleus, and nucleus accumbens shell (Saito et al. 2001).

The prominence of MCH1R in limbic brain regions has led to studies on the role of the MCH system in stress and anxiety. The present conclusions of these studies, however, are controversial. Central MCH injection has been reported to be either anxiogenic (Smith et al. 2006) or anxiolytic (Monzon and De Barioglio 1999; Monzon et al. 2001) in the elevated plus maze. Microinjection of MCH into the

nucleus accumbens shell, an area of the brain where MCH has been reported to potentiate dopamine signaling (Chung et al. 2009), promotes depression-like behavior (Georgescu et al. 2005). MCH1R knockout mice exhibit reduced anxiety (Roy et al. 2006; Smith et al. 2006) and depression-like behaviors (Georgescu et al. 2005) in several behavioral paradigms. Finally, several groups have used MCH1R antagonists to search for a link between the MCH system and anxiety. In 2002, the MCH1R antagonist SNAP-7941 was shown to produce anxiolytic effects in a social interaction test and to reduce separation induced vocalization in guinea pig pups (Borowsky et al. 2002). Additional MCH1R antagonists, SNAP 94847, ATC0065, ATC0175, and GW3430, were found to also be anxiolytic in acute and chronic anxiety behavioral paradigms (Shimazaki et al. 2006). However, it has also been reported that MCH1R antagonists are ineffective in anxiety paradigms (Basso et al. 2006) and that several of the MCH1R antagonists reported to be anxiolytic bind to targets other than MCH1R that could contribute to the observed anxiolytic effects (Basso et al. 2006), raising questions about whether inhibiting the MCH system is sufficient to produce anxiolytic effects.

The present study examined the anxiolytic effects of a MCH1 receptor antagonist, TPI 1361-17. This antagonist exhibits high affinity (nM) and is specific for the MCH1 receptor (Nagasaki et al. 2009). In contrast to previously reported MCH1R antagonists, TPI13161-17 does not cross the blood–brain barrier and is thus administered intracerebroventricularly (i.c.v.) which ensures that its central effects are not affected by peripheral MCH-related responses such as potential alterations in insulin levels (Pissios et al. 2007). TPI1361-17 was therefore tested on mice in two widely used behavioral assays for anxiety: the elevated plus maze and the light–dark transition paradigms.

Materials and Methods

Animals

Male C57BL/6 mice (National Cancer Institute, Bethesda, MD, USA; age, 7–8 weeks; weight, 23–25 g) were used in all experiments. The animals were group-housed (four animals per cage) under controlled conditions with a 12-h light–dark cycle and with ad libitum access to food and water. All animal experiments were approved by the University of California Irvine Institutional Animal Care and Use Committee.

Drugs

TPI1361-17 was dissolved in phosphate-buffered saline (pH 7.4) with 0.2% bovine serum albumin. Prior to i.c.v. injection, mice were briefly anesthetized with isoflurane and TPI1361-17 or vehicle was injected into the lateral ventricle (i.c.v.) with 2 μL total volume using a Hamilton syringe as described previously (Laursen and Belknap 1986; Xu et al. 2004). Mice were allowed to recover for 20 min prior to anxiety testing. Following completion of the experiment, mice were euthanized and brains examined to confirm correct injection site. Animals with an injection site not in the lateral ventricle were excluded from the study. Approximately 95% of injections were correctly placed.

Chlordiazepoxide (Sigma-Aldrich, St. Louis, MO, USA) was dissolved on the morning of the experiment in saline. Five milligrams per kilogram of chlordiazepoxide or saline was injected i.p. in a volume of 4 ml/kg 20 min prior to behavioral testing.

Elevated Plus Maze Paradigm

The elevated plus maze task was performed as described previously with minor modifications (Koster et al. 1999; Xu et al. 2004). Briefly, the plus maze consisted of two open (30×5 cm) and two wall-enclosed arms (30×5×15 cm) connected by a central platform (5×5 cm) in a dimly lit room. The apparatus was elevated 75 cm above the floor. Mice were transferred in their home cages to the behavioral testing room 1 h prior to the start of behavioral testing. The test was started by placing a mouse in the central area of the plus maze facing a closed arm which the animal usually enters first. Exploratory behavior was monitored using an automated video motility system (Video Mot II, TSE, Bad Homburg, Germany). The numbers of entries into open arms, time in open and closed arms, total number of zone transitions, and latency until the first open-arm entry were recorded and quantified. Entries were defined as the body center of an animal entering a new zone.

Light–Dark Transition Paradigm

The light–dark transition test was performed as described previously (Koster et al. 1999; Xu et al. 2004). The light–dark box was divided into a lit compartment (30×20×25 cm) and a dark compartment (15×20×25 cm) connected by a 4-cm tunnel in a dimly lit room. Mice were initially placed in the dark compartment. The number of entries into the light compartment, time in light compartment, and latency to enter light compartment during the 5-min test were recorded using the same program as was used for elevated plus maze experiments. Entries were defined as the body center of an animal entering a different compartment.

Statistics

All statistical analysis was performed using Graphpad Prism 5.0. Nonparametric variables such as the number of

entries into each arm were analyzed by Pearson's chi-square test. All other parameters were analyzed by Student's t test or one-way analysis of variance with Bonferroni post-test. In all experiments, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, and $^{\#}p<0.05$ vs 0.5 nmol TPI-1361-17 group.

Results

Light–Dark Transition Test

The light–dark transition test was validated with mice injected with 5 mg/kg chlordiazepoxide or saline. Mice administered chlordiazepoxide spent significantly more time in the light compartment (74.12 vs 41.78, $p<0.05$, Table 1), exhibited more entries into the light compartment (9.43 vs, 6.17 $p<0.05$, Table 1), and had a shorter latency to first entry into the light compartment (11.23 vs 48.76, $p<0.05$, Table 1) than vehicle injected control mice, indicating an anxiolytic effect.

A separate group of mice was injected i.c.v. with either 0.5 or 1 nmol of TPI 1361-17 or vehicle and tested in the light–dark transition test. Mice injected with 1 nmol TPI 1361-17 spent significantly more time in the light compartment (66.12 vs 32.02, $F_{2,27}=9.73$, $p<0.01$, Fig. 1a), entered the light compartment more times (8.3 vs 5.6, $F_{2,27}=3.55$, $p<0.05$, Fig. 1b) and had a shorter latency to first entry into the light compartment (16.46 vs 39.25, $F_{2,27}=3.66$, $p<0.05$, Fig. 1c) than vehicle-injected animals, and also spent significantly more time in the light compartment than mice injected with 0.5 nmol TPI 1361-17 (66.12 vs 45.19, $F_{2,27}=9.74$, $p<0.05$, Fig. 1a). Mice injected with 0.5 nmol TPI 1361-17 exhibited trends toward reduced anxiety like behaviors (Fig. 1).

Elevated Plus Maze

To validate the assay, mice were injected with 5 mg/kg i.p. chlordiazepoxide or saline and tested on the elevated plus maze. Mice administered chlordiazepoxide showed a significant increase in time spent on the open arms (42.78 vs 20.44, $p<0.05$, Table 1), and the number of entries into open arms (9.00 vs 4.78, $p<0.05$, Table 1), and exhibited a strong trend towards a shorter latency to first entry onto an open arm (Table 1).

A separate group of mice was injected i.c.v. with 1 nmol TPI 1361-17 or vehicle and tested on the elevated plus maze. Mice injected with TPI 1361-17 spent more time on the open arms of the plus maze (38.9 vs 6.99, $p<0.01$, Fig. 2a), and entered the open arms more frequently (7.6 vs 5.0, $p<0.05$, Fig. 2b) than vehicle-injected animals. TPI 1361-17 injected mice trended toward a shorter latency to first open arm entry (Fig. 2c).

Discussion

The elevated plus maze and light–dark transition tests are commonly used mouse models of anxiety-like behavior. Compounds with anxiolytic effects cause mice to spend more time in and enter more frequently the open arms of the elevated plus maze or the lit compartment of the light–dark box (Lister 1987; Bourin and Hascoet 2003). In the present study, we first used the benzodiazepine chlordiazepoxide to validate two assays, the light–dark transition test and the elevated plus maze paradigms, and then demonstrated that the selective MCH1R antagonist TPI 1361-17 exerts potent anxiolytic effects in these models.

There was no observed effect on the total number of transitions between zones in the elevated plus maze (15.40±2.056 vs 17.90±1.224), indicating that central administration of TPI 1361-17 is not sedative, which is consistent with a previous report that TPI 1361-17 does not alter locomotor activity (Nagasaki et al. 2009). The fact that TPI 1361-17-injected mice spend more time in the non-protected zones of both assays indicates that TPI 1361-17 exerts anxiolytic effects. A dose-dependent relationship is present for anxiolytic effects in the light–dark transition assay. Although it is not possible to accurately compare the magnitude of the anxiolytic effects of chlordiazepoxide and TPI 1361-17 as presented in this study due to

Table 1 Validation of light–dark transition test and elevated plus maze

	Light–dark transition test		Elevated plus maze	
	Vehicle	Chlordiazepoxide	Vehicle	Chlordiazepoxide
Time spent in open arms (s)	41.78±7.29	74.12±10.13*	20.44±3.44	42.78±7.67*
Entries into light compartment/open arms	6.167±0.87	9.43±0.72*	4.78±0.57	9.00±0.65***
Latency to first entry into light compartment/open arms	48.76±14.90	11.23±2.94*	37.38±7.27	20.21±5.25

The effects of 5 mg/kg i.p. chlordiazepoxide on time spent in the light compartment/open arms, number of entries into the light compartment/open arms, and the latency to first entry into the light compartment/open arms in the light–dark transition test and elevated plus maze

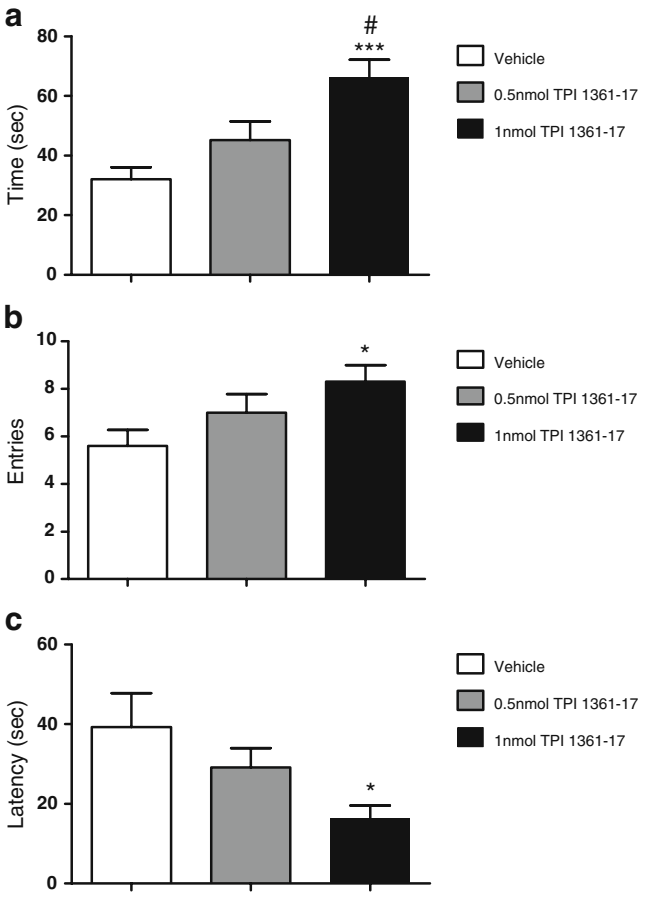

Figure 1 Light–dark transition test. The effects of i.c.v. injection of vehicle, 0.5 nmol TPI 1361-17, 1 nmol TPI 1361-17 on **a** time spent in the light compartment, **b** number of entries into the light compartment, and **c** and latency to first entry into light compartment (N=9–10 animals per group)

differences in experimental procedure, i.c.v. injection of TPI 1361-17 causes a reduction of anxiety-like behavior that appears similar to that observed with 5 mg/kg chlordiazepoxide i.p. in both behavioral paradigms, indicating that the inhibition of the MCH system has a potent effect on anxiety-like behavior.

TPI 1361-17 is a highly specific MCH1R antagonist that inhibits its receptor at nanomolar concentrations and does not display activity or binding to any targets tested in an array of G-protein protein coupled receptors and channels (Nagasaki et al. 2009). TPI 1361-17 has been shown to be effective in vivo, since it blocks MCH-induced food intake (Nagasaki et al. 2009) and inhibits acute cocaine self-administration (Chung et al. 2009). The high degree of specificity indicates that the anxiolytic effects of TPI1361-17 are entirely due to blockade of the MCH system and that off-target effects are unlikely to contribute to the observed effect. Central administration of TPI 1361-17 eliminates the possibility of any peripheral effects of the compound contributing to the anxiolytic effects. This evidence supports MCH involvement in the regulation of anxiety and stress response (Smith et al. 2006, 2009) and is consistent with previous reports indicating that MCH1R antagonists are anxiolytic and may be useful in treating anxiety disorders.

The mechanisms underlying the anxiolytic activity of MCH antagonists are unknown, but several lines of evidence indicate that multiple factors may contribute to this effect. MCH has been reported to modulate the hypothalamic–pituitary adrenal axis (Smith et al. 2006, 2009), possibly by activating neurons in the paraventricular nucleus and increasing ACTH and corticosterone release (Herman et al. 1996; Smith et al. 2006), suggesting that MCH may regulate anxiety and stress response at least partially through this circuit. It has also been reported that microinjection of MCH into the nucleus accumbens shell induces depressive-like effects (Georgescu et al. 2005), and it was recently discovered that MCH modulates dopamine signaling in this region (Chung et al. 2009), suggesting that the effects of MCH on depression-like behavior may involve the dopamine system. Anxiety and depressive

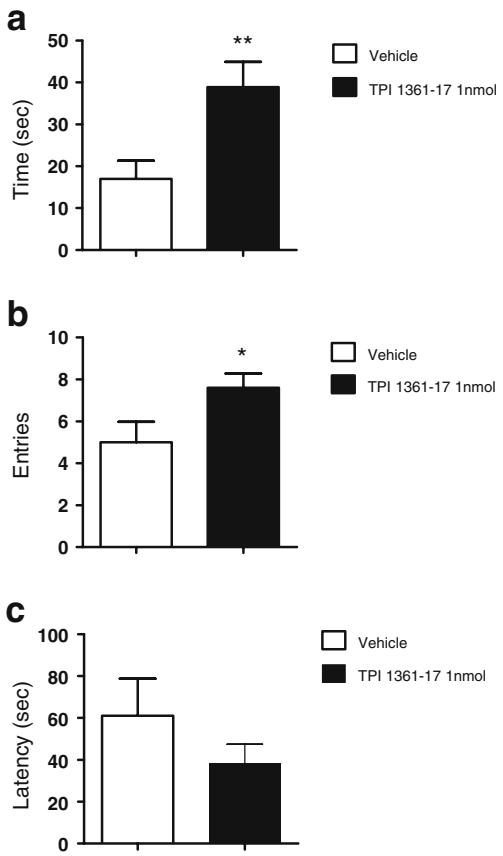

Figure 2 Elevated plus maze. The effect of i.c.v. injection of vehicle or 1 nmol TPI 1361-17 on **a** time spent on the open arm, **b** number of entries onto the open arm, and **c** Latency to first entry onto an open arm (N=10 animals per group)

disorders are closely linked and express with high comorbidity (Dunlop and Davis 2008), suggesting that the anxiolytic and antidepressant effects of MCH antagonists could occur through similar mechanisms and that the role of MCH in anxiety could thus also involve the mesolimbic dopamine system. Although little is known about the role of MCH in the amygdala, MCH projects to (Bittencourt et al. 1992) and MCH1R is highly expressed in several nuclei of the amygdala (Saito et al. 2001), in areas in which other neuropeptide systems are known to regulate anxiety (Tasan et al. 2010).

We have shown that acute central administration of the specific MCH1R antagonist TPI 1361-17 exerts potent anxiolytic effects in two of the most common and accepted anxiety behavioral paradigms. The high specificity of TPI 1361-17 indicates that its anxiolytic effects are caused by blockade of MCH1R alone. These results support previous studies indicating that MCH1R is a viable target for the development of novel anxiolytic drugs and suggests that the reported anxiolytic effects of other MCH1R antagonists are caused by blockade of the MCH system and are not due to off target effects.

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