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Mice Lacking Melanin Concentrating Hormone 1 Receptor are Resistant to Seizures

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Abstract

The Melanin Concentrating Hormone (MCH) system is widely expressed throughout the central nervous system and regulates a variety of physiological functions. It has been reported that acute central administration of MCH inhibits pentylenetetrazol (PTZ)-induced seizures in rats. In the present study MCH₁ receptor knockout mice (MCH₁R-KO) were used to investigate the role of MCH signaling in modulating seizure susceptibility. Seizure behaviors were compared between MCH₁R-KO and wild type (MCH₁R-WT) mice following administration of the convulsant compounds PTZ or pilocarpine. PTZ injection induced clonic seizures in MCH₁R-WT mice but failed to induce them in MCH₁R-KO mice. More than twice as many injections of intermittently administered low dose PTZ were required to induce clonic seizures in MCH₁R-KO mice than in MCH₁R-WT mice. Following pilocarpine injection, MCH₁R-WT mice experienced clonic seizures and most had tonic seizures and entered status epilepticus, while all MCH₁R-KO mice were completely resistant to these effects. MCH₁R-KO mice were also observed to be strongly protected from the development of PTZ kindling. Genetic deletion of MCH₁R conferred resistance to all seizure models tested in this study. The data indicate that the MCH system is involved in the regulation of PTZ and pilocarpine seizure threshold.

Introduction

Melanin Concentrating Hormone (MCH) is a cyclic neuropeptide produced primarily in the lateral hypothalamus and zona incerta that activates one G protein-coupled receptor in rodents, the Melanin Concentrating Hormone 1 receptor (MCH₁R) [18]. Studies have strongly indicated that MCH may regulate food intake [15], anxiety-like behavior [19], depression-like behavior [3], performance in learning and memory behavioral tests [21], and drug self administration [5] in rodents. MCH₁R is expressed throughout the brain with particularly dense localization in the nucleus accumbens shell, hippocampus, amygdala, locus coeruleus and cerebral cortex [17] which suggests the receptor is involved in a wide range of physiological functions

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In 1997 Knigge et al reported that acute central injection of MCH inhibits pentylenetetrazol (PTZ)-induced seizures in both rats and guinea pigs [11], but this finding has not been further investigated. The strong anticonvulsant effect observed with central MCH administration suggests that MCH may act as an endogenous modulator of seizure activity. MCH projects strongly to several brain regions with known involvement in epileptic seizures [2]. MCH action in the hippocampus enhances memory retention [21], and increases synaptic transmission [22] through mechanisms involving NMDA signaling [23], but little else is known about MCH function in this and other brain areas involved in seizures. In order to gain insight into the involvement of the endogenous MCH system in modulating seizure threshold we have compared the seizure susceptibility of MCH₁R-KO and WT mice in several distinct seizure models: acute PTZ seizure, pilocarpine induced seizure, and PTZ kindling. In light of the reported anticonvulsant effects of central MCH injection [11], we hypothesized that MCH₁R-KO would exhibit increased susceptibility to seizures.

Materials and Methods

Animals

Melanin Concentrating Hormone receptor knockout mice (MCH₁R KO) were obtained from Su Qian, Merck Inc. Mice had been backcrossed onto a C57/Bl6 background for at least 9 generations. The homozygous progeny of MCH₁R heterozygote X heterozygote matings were used to generate WT and MCH₁R KO lines. The F1, F2, and F3 generations of these lines were used in all experiments. Both male and female mice were used. All mice were 7–10 weeks of age at the beginning of experiments and each mouse was used in only one seizure paradigm. Each animal was used in only one seizure paradigm. All mice were group housed under a 12hr light/dark cycle with ad libitum access to standard lab chow and water. All experimental procedures were performed in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the local Institutional Animal Care and Use Committee.

Drugs

Pentylenetetrazol, pilocarpine hydrochloride, and scopolamine methyl bromide (Sigma-Aldrich) were dissolved in 0.9% NaCl, and injected in a volume of 4ml/kg.

Seizure Scoring

Seizures in all paradigms were scored according to a modified Racine scale [16] as follows: A stage 1 seizure corresponds to a cessation of movement and grooming behavior, a stage 2 seizure is a forelimb and/or tail extension with rigid posture or shaking behavior, a stage 3 seizure is the appearance of myoclonic jerks of the head and neck, a stage 4 seizure is forelimb clonus and partial rearing, a stage 5 seizure is forelimb clonus with falling, and in a stage 6 seizure mice jump uncontrollably and show tonic seizure with hindlimb extension which often results in death. Status epilepticus is unremitting seizures.

Acute PTZ Seizure

Mice were injected with 50mg/kg PTZ via intra-peritoneal injection (i. p.) and were monitored in individual plastic cages for 20 minutes by a trained observer. Latency to each seizure behavior was scored. If a mouse did not progress to a given seizure behavior, latency was scored as 1200 seconds.

Intermittent PTZ Administration

Intermittent PTZ administration experiments were performed as previously reported with minor modifications [10]. Mice were initially injected with 20mg/kg PTZ subcutaneously (s.c.). Every 10 minutes mice were administered an additional dose of 10mg/kg PTZ. Mice were monitored in individual plastic cages and the highest seizure score observed in each 10 minute bin was recorded. The number of additional injections required for induction of the first stage 5 clonic seizure and also for a second stage 5 clonic seizure that generally progressed to tonic seizure and status epilepticus were recorded. Mice which progressed to tonic seizure or status epilepticus after their first seizure were recorded as requiring the same number of injections to induce the first and second seizures.

PTZ kindling

A sub-convulsive dose of PTZ, 30mg/kg s.c., was injected every other day. A trained observer monitored mice for 30 minutes following injection and the highest seizure score observed was recorded for each session. Mice were considered kindled once a stage 5 seizure was observed on consecutive sessions. Once a mouse became kindled, it was removed from the study and scored as a stage 5 seizure in all subsequent sessions.

Pilocarpine Seizures

30 minutes prior to pilocarpine injection, mice were injected with 1mg/kg s.c. scopolamine methyl bromide to reduce peripheral muscarinic effects [4]. Mice were injected with 350 mg/kg i.p. pilocarpine hydrochloride and monitored in a clear plastic cage for 2 hours by a trained observer. The maximal seizure behavior observed in each 5 minute time bin was recorded.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5.0 software. Two way analysis of variance (ANOVA) was performed to determine differences between genotypes for acute and intermittent PTZ seizure and pilocarpine seizure models. An unpaired student's t test was used to determine differences in average seizure score at individual timepoints in the PTZ kindling experiment. Nonparametric variables such as the number or percentage of mice exhibiting a given seizure stage were analyzed using Fisher's exact test. Kaplan-Meier survival analysis was performed for PTZ kindling experiments. In all experiments * corresponds to a p value of <0.05, ** to <0.01, and *** to <0.001.

Results

Injection of 50mg/kg i.p. of PTZ induced behavioral arrest and myoclonic jerks that progressed to forelimb clonus and stage 5 clonic seizures in all wild type mice tested. In contrast, mice lacking the Melanin Concentrating Hormone 1 receptor (MCH₁R-KO) were highly resistant (Table 1) to PTZ-induced seizures. MCH₁R-KO mice exhibited behavioral arrest and a rigid posture with a similar latency as wild type mice (Fig 1A), but significantly fewer (Table 1) MCH₁R-KO mice displayed mild stage 3 myoclonic jerks (18% vs 100%, $p<0.001$) and none exhibited forelimb clonus or progressed to clonic seizure (0% vs 100%, $p<0.0001$). The average latency to stage 3, stage 4, and stage 5 seizures were significantly different ($p<0.001$) between the two genotypes.

Intermittent administration of low doses of PTZ produced an array of seizure behavior similar to that exhibited in the acute PTZ administration model. Wild type mice progressed from behavioral arrest and myoclonic jerks to a single clonic seizure and later exhibited a second episode of clonic seizures that usually resulted in status epilepticus, or death. Mice lacking MCH₁R required significantly more injections (Fig 1B, $p<0.001$) of 10mg/kg PTZ

following the initial PTZ administration than did wild type mice to progress to (Fig 1B) myoclonic jerks (7.33 vs 3, $p < 0.001$) and to their first (11.0 vs 3.33, $p < 0.001$) and second clonic seizures (11.17 vs 5.17, $p < 0.001$). Interestingly, MCH₁R-KO mice often progressed to higher order seizure behaviors (status epilepticus, tonic seizure) immediately following their first clonic seizure. The magnitude of the difference in PTZ doses required to elicit clonic seizures indicates that MCH₁R-KO mice have a much higher PTZ induced seizure threshold than WT mice.

Injection of 350mg/kg of pilocarpine caused a different array of seizure behaviors compared to those observed with PTZ treatment. Shortly after injection, all wild type mice displayed behavioral arrest and tremors of increasing intensity that progressed to myoclonic jerks, forelimb clonus, and clonic seizures, typically culminating in status epilepticus and/or tonic seizures and death. There was no difference between genotypes for expression of behavioral arrest and body tremors following pilocarpine administration but profound differences were observed for all other seizure behaviors (Fig 2). All MCH₁R-WT mice injected with pilocarpine had at least one clonic seizure, 90% of which entered status epilepticus, while 70% progressed to tonic seizure and 60% died during the two hour observation period (Fig 2). Conversely, MCH₁R-KO mice were completely resistant to forelimb clonus (0% vs 100%, $p < 0.0001$, data not shown), clonic seizures with loss of balance (0% vs 100%, $p < 0.0001$), tonic seizures (0% vs 70%, $p < 0.01$), status epilepticus (0% vs 70%, $p < 0.01$), and death (0% vs 60%, $p < 0.05$) (Fig 2).

Genetic inactivation of MCH₁ receptors almost completely blocked development of seizures in a PTZ kindling experiment. Clonic seizures began to develop by the sixth PTZ injection session (Fig 3) and eventually occurred in all MCH₁R-WT mice. In contrast, only 23% of MCH₁R knockout mouse experienced clonic seizures in any session. Knockout mice developed advanced seizure behavior much more slowly than wild type mice (Fig 3), and most did not progress past stage 3 seizures at any point in the study. The average seizure scores of the two genotypes were significantly different by session 5 ($p < .05$), and became more divergent throughout the course of the experiment. The median time to kindling for WT mice was 11 sessions and all WT mice became kindled by session 18 (Fig 4). In contrast, no MCH₁R-KO mice were kindled at session 11 and only two became kindled at any point in the study (Fig 4, $n=13$). 85% of MCH₁R-KO mice were completely protected from PTZ kindling (Fig 4). A Kaplan-Meier survival analysis (Fig 4) showed that mice without MCH₁ receptors required significantly longer ($p < .0001$) to become kindled than wild type mice, indicating that MCH₁R-KO mice are highly resistant to PTZ kindling.

Discussion

This study demonstrates that mice lacking the MCH₁ receptor are resistant to pentylenetetrazol and pilocarpine-induced seizures and are much less susceptible to PTZ kindling than wild type mice. Though littermates from heterozygous breeding pairs were not compared, the homozygous breeding method used in this study produces mice that are closely related, indicating that differences between the two genotypes can be directly compared and are unlikely to be confounded by subtle differences in genetic background. The presentation of seizure behaviors did not appear qualitatively different between the two genotypes, which suggesting that the seizure resistance observed is due to an enhanced seizure threshold in MCH₁R knockout mice and is not caused by alterations in response to PTZ or pilocarpine. The fact that seizure resistance is observed in four separate experimental paradigms suggests that inactivation of the MCH signaling system decreases susceptibility to several types of seizures.

It has been reported that acute central injection of MCH has an anticonvulsant effect on pentylenetetrazol-induced seizures in rats [11]. Consequently, it would be expected that mice lacking the MCH₁ receptor would be more susceptible to chemically induced seizures, a hypothesis which is not supported by our data. While it is possible that this is a result of species differences, it is more likely that other factors account for this discrepancy. We have used mice in which MCH₁R signaling has been chronically inactivated while the Knigge et al [11] study examined the acute effects of central MCH administration. MCH₁R couples to G_i [7] and is highly expressed in brain regions involved in seizure activity [1,9,17], thus the acute anticonvulsant effects of MCH could be explained by an acute inhibitory effect on neuronal firing in these brain regions. MCH administration has been reported to positively regulate NMDA receptor expression in the hippocampus [23]. Thus while acute MCH may have an immediate inhibitory effect on seizure activity, it leads to alterations in NMDA receptor expression. NMDA receptor signaling has long been thought to mediate aspects of epileptic seizures [1,13], so MCH mediated alterations in NMDA receptor expression could in turn lead to a net long term proconvulsant role for MCH. Thus, endogenous MCH could in fact lower seizure threshold. This phenomenon of “effect inversion” where the effects of acute administration of an agonist or antagonist/inverse agonist are opposite those seen with chronic administration is well documented for adenosine [8,24,25] and beta-adrenergic receptors [8,14]. Interestingly, one behavior in which this “effect inversion” has been observed for chronic vs acute adenosine A₁ receptor agonism and antagonism is in the regulation of seizures [8,25].

The mechanism by which MCH regulates seizure threshold is unknown. Clonic seizures induced by pentylenetetrazol are believed to involve the cerebral cortex and forebrain [6] and pilocarpine is a model for temporal lobe epilepsy [20] that causes extensive damage to the hippocampus. These areas, and other brain regions commonly involved in epilepsy such as the amygdala, receive extensive MCH projections and express high levels of MCH₁R [2,17]. The functions of MCH in the frontal cortex, amygdala, and hippocampus are not well understood, but the correlation between MCH₁R distribution and seizure focal point implies that MCH may affect seizure threshold in these brain areas. It is unknown whether MCH₁R-KO mice are resistant to seizures originating in brain areas that do not normally express high levels of MCH₁R. This could be investigated using an alternate seizure model in which seizures originate in brainstem structures such as electroshock seizures [6].

This study provides evidence that mice lacking the MCH₁ receptor are highly resistant to both acute and chronic seizure models. Though there are limitations involved in the use of knockout mice and we cannot completely exclude the involvement of compensatory developmental effects, this data combined with the previous demonstration of the effects of MCH administration on PTZ seizure [11] provide strong evidence for the involvement of MCH in modulating seizure threshold. Little is known about how MCH may regulate seizure threshold, but correlative neuroanatomical evidence suggests that it may involve the hippocampus, cortex, and amygdala, but no studies have yet provided substantial evidence for this hypothesis. Genetic inactivation of MCH₁R may mirror what would occur with long-term pharmacological inactivation of the receptor, suggesting that MCH₁R antagonists may be effective in the treatment of epilepsy. This is particularly noteworthy as the strong resistance of MCH₁R knockout mice to pilocarpine induced seizures implicates MCH₁R antagonists as a potential treatment for temporal lobe epilepsy which is known to be particularly refractory to current pharmacological treatments [12].

Research Highlights

- MCH₁R-KO mice are resistant to PTZ induced myoclonic jerks and clonic seizures

- MCH₁R-KO mice are resistant to the intermittent low dose PTZ
- MCH₁R-KO mice are highly resistant to pilocarpine induced seizures
- MCH₁R-KO mice are resistant to the development of PTZ induced kindling.

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Fig 1.

Comparison of the convulsant effects of PTZ on MCH1R-KO and MCH1R-WT mice (A) Average latency to the onset of specific seizure behaviors following injection of 50mg/kg PTZ (i.p.). N=9 WT, 11 MCH1R KO (B) The number of s.c. injections of 10mg/kg PTZ administered every 10 minutes required to induce myoclonic jerks and the first and second clonic seizures following an initial loading dose of 20mg/kg PTZ. N= 6 per genotype □= MCH1R WT, ■=MCH1R KO

Fig 2.

Comparison of the percent of mice displaying seizure behaviors in the two hours following i.p. injection of 350mg/kg pilocarpine. N= 10 for each genotype. "S.E." = status epilepticus.

□= MCH₁R WT, ■=MCH₁R KO

Fig 3.
The average Racine seizure score of mice observed during each session of PTZ kindling.
N=13 for each genotype. □= MCH₁R WT, ■=MCH₁R KO

Fig 4.

Kaplan-Meier survival analysis for PTZ kindling experiment. Displayed is the percentage of mice protected from entering a kindled state, which is defined as the occurrence of clonic seizures on two consecutive sessions. MCH1R-KO were significantly ($P < 0.0001$; logrank Mantel-Cox test) less likely to become kindled than MCH1R-WT mice. $\square$ = MCH₁R WT, $\blacksquare$ = MCH₁R KO

Table 1

Proportion of mice that displayed each seizure behavior in the 20 minute observation period following i.p. injection of 50mg/kg PTZ

Genotype	Seizures observed following 50mg/kg PTZ					
	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
MCHIR +/+	9/9	9/9	9/9	9/9	9/9	0/9
MCHIR -/-	11/11	11/11	2/11 ***	0/11 ***	0/9 ***	0/11