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γ1- and γ2-melanocyte stimulating hormones induce central anxiogenic effects and potentiate ethanol withdrawal responses in the elevated plus-maze test in mice

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ABSTRACT

Little is known about the endogenous functions of γ1- and γ2-melanocyte stimulating hormones (γ1- and γ2-MSH). Although γ-MSHs bind to melanocortin receptor subtypes 3 and 4, we have previously shown that these peptides also influence non-melanocortinergic processes, such as dopaminergic and GABAergic. The aim of this study was to determine the effects of γ1- and γ2-MSH (at doses 0.3, 1 and 2 nmol/mouse/5 μl) on the anxiety levels in mice in elevated plus maze. Three experimental paradigms were performed to assess the effects of peptides on: a) ethanol withdrawal; b) acute ethanol-induced anxiolytic action; c) peptides *per se*. We used ethanol as the model substance, since its action involves either dopaminergic/GABAergic or melanocortinergic processes. γ-MSHs were administered intracisternally in mice and behavioural responses were assessed in the elevated plus maze test. This study provides the first demonstration of an anxiogenic effect of γ1- and γ2-MSH, their synergistic/additive effect on ethanol withdrawal-induced anxiety behaviour, and an antagonism of peptides involved in the anxiolytic action of ethanol. Furthermore, results suggest that γ-MSHs belong to an anxiogenic peptide family that may play an important role in anxiety disorders as well as in the development of alcohol dependence and/or alcohol withdrawal-induced behaviours.

1. Introduction

The discovery of five subtypes of melanocortin receptors (MCR1-5) in the early 1990s was followed by an avalanche of studies that have provided additional insights on the physiological roles of melanocortins – α-MSH, γ1-MSH and γ2-MSH (i.e., melanocyte stimulating hormones, or MSHs) – and their receptors. Among all MSHs, α-MSH has become the most studied peptide due to its high binding affinity to MCR1 and MCR3-5 (Schioth et al., 1995). A broad spectrum of α-MSH peripheral and central effects, such as stimulation of melanogenesis, anti-inflammatory action, influence on behaviour and learning ability, and pain perception have been demonstrated (for reviews see, Wikberg, 1999; Wikberg et al., 2000; Schioth, 2001).

Of all the melanocortins, the functions of γ-MSHs are least known. Radioligand receptor binding studies have shown a high binding affinity of γ-MSHs for MCR3 and MCR4 (Lindblom et al., 1998). These receptor subtypes are abundantly expressed in brain structures that are part of the dopaminergic mesolimbic system (*ventral tegmental area*, VTA; *nucleus accumbens*, NACC) (Roselli-Rehfuss et al., 1993), which is considered a crucial system for the regulation of motivational behaviour and reward processes (Spanagel and Heilig, 2005).

Our earlier work suggested that both γ-MSHs are involved in the regulation of emotional homeostasis provided by the dopaminergic mesolimbic system, since both peptides influenced behavioural responses when introduced into the VTA of rats (Klusa et al., 1999) and changed the levels of dopamine (DA) and its metabolite DOPAC in the NACC of these animals (Jansone et al., 2004). Other data obtained in mice has demonstrated that γ1-MSH (injected intracisternally) significantly attenuated analgesia caused by ethanol and diazepam (both GABA-A receptor ligands), while γ2-MSH did not change diazepam effects but considerably potentiated ethanol-induced analgesia (Klusa et al., 2001). Interestingly, α-MSH caused a short hyperalgesic effect, while γ1-MSH induced short-term analgesia and γ2-MSH caused a stable and markedly expressed central analgesia. γ2-MSH-induced analgesia was antagonized only by the GABA-A receptor antagonist bicuculline, and not by HS014 (a melanocortin MC3/MC4 receptor antagonist) or the opiate receptor antagonist naloxone.

Our earlier results clearly indicate that γ-MSH peptides influence certain non-melanocortinergic mechanisms, particularly those acting via the DA receptor and the GABA/benzodiazepine/Cl^- receptor complex. Particular interest has been focused on data showing the ability of γ-MSHs to influence ethanol-induced analgesia (Klusa et al., 2001). First, ethanol is known as a complex modulator of the chloride channel of the GABA-A receptor (Aguayo et al., 2002; Allan et al., 2008). It increases GABA-A receptor-mediated inhibition, and chronic ethanol administration results in tolerance, dependence and ethanol withdrawal syndrome, which are mediated in part by the desensitization of GABA-A receptors (Faingold et al., 1998). Second, GABA-A receptors appear to play a complex role in the mediation of drug reinforcement, motivational behaviour and reward processes. These depend on the dynamic functional state of GABA-A receptors in both

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DA and non-DA neurons (Xi and Stein, 1998; Spanagel and Heilig, 2005). Third, radioligand receptor binding studies have shown that γ-MSHs have a high binding affinity to MCR3 and MCR4 (Lindblom et al., 1998), the subtypes that are abundantly expressed in the brain structures associated with the dopaminergic mesolimbic system (Roselli-Rehfuss et al., 1993). Finally, in some brain structures (e.g., the nucleus arcuatus) GABA is co-expressed with pro-opiomelanocortin, a precursor of biodegradation that leads to the formation of MSH peptides (Naftolin et al., 1990). Since ethanol administration is well known to cause anxiolytic effects in rodents (Kameda et al., 2007) and its withdrawal leads to elevated anxiety levels (Doremus et al., 2003), it was of interest to test the influence of γ-MSH peptides on ethanol effects to investigate the role these peptides play in the neurocircuitry involved in regulating ethanol-induced behaviours.

Recent data has demonstrated the ability of α-MSH to suppress the anxiolytic behavioural effects caused by ethanol and to block ethanol withdrawal anxiety by HS014 (Kokare et al., 2006). Furthermore, α-MSH has been shown to demonstrate anxiogenic activity in the elevated plus maze test (Gonzalez et al., 1996; Vecsernyes et al., 2000; Kokare et al., 2006). It is therefore suggested that the melanocortin system is implicated in ethanol-induced effects (Kokare et al., 2006), although it remains to be established whether or not α-MSH acts as a unique peptide from the MSH peptide family, or whether other MSHs, such as γ-MSHs, can also contribute as functional groups capable of modulating ethanol-induced behaviour.

The primary aim of this study was to determine the effects of γ1- and γ2-MSH (at doses 0.3, 1 and 2 nmol/mouse/5 μl) on the anxiety levels in mice. Three experimental paradigms were performed to assess the effects of peptides on: a) ethanol withdrawal; b) acute ethanol-induced anxiolytic action; c) peptides *per se*. Behavioural responses of mice were assessed using the elevated plus maze test (Pellow and File, 1986) after intracisternal administration of γ-MSHs. The elevated plus-maze test has been established as that to evaluate anxiety related behaviour patterns in rodents (Pellow and File, 1986). This test was performed also in experiments with α-MSH by other authors (Kokare et al., 2006) that may give a comparative insight in MSH peptides activities. To distinguish whether decreased locomotion in the elevated plus maze was reflective of anxiety or catalepsy, a test for catalepsy was carried out immediately after the plus maze procedure in all experiments.

2. Methods

2.1. Animals

Male Icr:Icl mice (from the Riga Stradins University, Riga, Latvia), weighing 20–23 g, were housed ten per cage. The experimental room was maintained under controlled temperature (21–23 °C) with lights on from 0700–1900 h. Food and water were available ad libitum. All experimental procedures were carried out in accordance with guidelines of the Directive 86/609/EEC "European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes" (1986) and were approved by the Animal Ethics Committee of the Food and Veterinary Service (Riga, Latvia).

2.2. Drugs

Peptides γ1- and γ2-MSH (BACHEM, Germany) were dissolved in artificial cerebrospinal fluid (aCSF). Ethanol solution (10% v/v) was prepared from 95% ethanol in sterile saline and administered via intraperitoneal (i.p.) injection.

2.3. Intracisternal injection and elevated plus maze test

Both γ1- and γ2-MSH were used at doses of 0.3, 1 and 2 nmol/mouse/5 μl and injected intracisternally (i.c.) (i.e., into *cisterna magna*) in conscious mice via a J-shape needle connected to a Hamilton syringe (Takagi et al., 1979). Animals of each experimental group ($n=10$) were housed in separate (isolated) pre- and post-injection cages. The behavioural effects were assessed in the elevated plus maze test between 0900 and 1400 h and the order of testing was counterbalanced across all treatment groups.

The elevated wooden plus maze, painted black, consisted of two opposite facing open arms and two opposite facing enclosed arms and had a common central platform (5×5 cm). The arms were 30 cm long, 5 cm wide and 50 cm above the floor. Closed arms had 15 cm high walls with an open roof. The testing room was illuminated by a 40 W red light bulb in the centre of the room. The experiment was begun by placing the mouse onto the central platform of the plus maze facing one of the open arms (Pellow and File, 1986). The time spent in open and closed arms and the central platform, as well as the number of entries into open and closed arms, was recorded on a Psion Workabout microcomputer (Noldus, Netherlands) for 5 min for each animal by the experimenter who present in the same room.

Arm entries were registered when all four paws of the animal were placed in one arm of the plus maze whereas an arm exit was scored when all four paws of the animal were not present in the arm. After each trial, the apparatus was cleaned with damp cotton and dried.

2.4. Experiment 1: assessment of behavioural effects of γ1- and γ2-MSH in the elevated plus maze test in naïve mice

Animals ($n=70$) were divided into seven groups (ten per group). Mice received γ1- or γ-2-MSH (0.3, 1 and 2 nmol/mouse/5 μl, i.c.) 20 min prior to testing in the elevated plus maze test. For the control group, mice received aCSF i.c. (5 μl). Behaviour was assessed for 5 min.

2.5. Experiment 2: assessment of the influence of γ1- and γ2-MSH on ethanol withdrawal-induced anxiety in the elevated plus maze test in mice

Animals ($n=80$) were divided into eight groups (ten per group). Seven experimental groups received ethanol at the dose of 2 g/kg i.p. for 10 days. A control group was given saline i.p. for 10 days (adapted from Cole et al., 2000). Ethanol or i.p. saline injection was stopped on day 10. Peptides (0.3, 1 and 2 nmol/mouse/5 μl) or aCSF (for aCSF and ethanol control) were injected i.c. on day 12 (i.e., on the 2nd day after discontinuation of the forced alcoholization), and animals of each treatment group were placed into a separate post-injection cage for 20 min. Plus maze behaviour was assessed for 5 min on day 12 following i.c. injections of peptide or aCSF.

2.6. Experiment 3: assessment of the influence of γ1- and γ2-MSH in acute ethanol-induced anxiolysis in the elevated plus maze test in mice

Animals ($n=80$) were divided into eight groups (ten per group). All experimental groups received ethanol (2 g/kg) i.p. 45 min before testing in the elevated plus maze. Control mice received saline i.p. After receiving ethanol or saline i.p., animals were placed into a separate post-injection cage. Twenty minutes prior to the testing, mice received γ1- and γ2-MSH (0.3, 1 and 2 nmol/mouse/5 μl, i.c.) or aCSF i.c. (5 μl) and were moved into a separate post-injection cage. Plus maze behaviour was then assessed for 5 min.

2.7. Catalepsy test

The catalepsy test (according to Kobayashi et al., 1997) was performed immediately after the plus maze procedure in all three experiments by placing both forepaws of the mouse over a horizontal bar (diameter 0.2 cm), elevated 15 cm from the surface of the desk. Time (s) for which animal maintained this position was recorded.

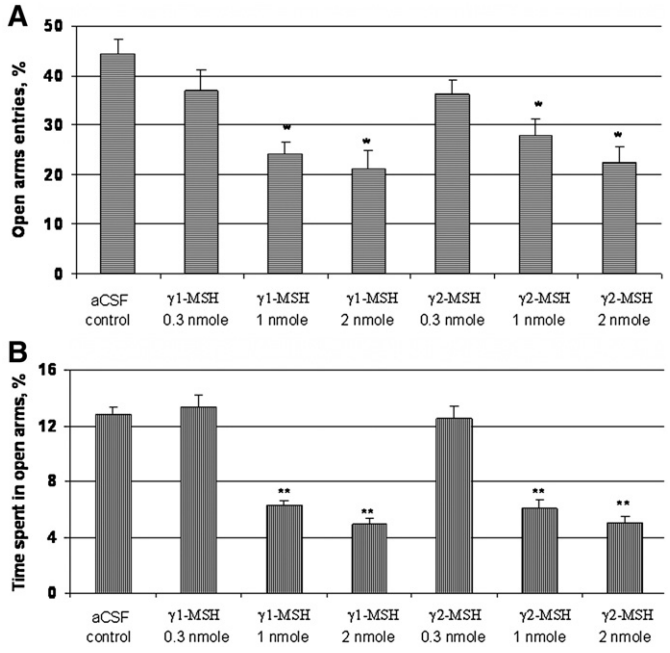

Fig. 1. Effects of γ1- and γ2-MSH (both at the doses of 0.3, 1 and 2 nmol/mouse/5 μl) on the exploratory behaviour. The percentage of open arms entries (A) and percentage of time spent in open arms (B) were observed on the elevated plus maze following 20 min i.c. administration of peptides or aCSF (5 μl). Values are mean±SEM. $n=10$ mice/group. Significance was measured by the one-way analysis of variance (ANOVA) followed by Dunnet's post-hoc test. *$P<0.05$; **$P<0.01$ vs. aCSF control group.

2.8. Statistics

All data are presented as mean±SEM. Statistical analysis was performed using the one-way analysis of variance (ANOVA) followed by Dunnet's post-hoc test. Differences were considered significant at the $P<0.05$ level. All statistical analyses were performed using the GraphPad Prism v. 4.00 (GraphPad Software, Inc.).

3. Results

3.1. Effects of γ-MSH peptides in the elevated plus maze test in naïve mice

Intracisternal administration of both γ1- and γ2-MSH at the doses of 1 and 2 nmol/mouse significantly reduced the percentage of open arm entries [$F(3.36)=7.44$, $P<0.001$ and $F(3.36)=5.54$, $P<0.005$] and the percentage of time spent in open arms [$F(3.36)=47.55$, $P<0.001$ and $F(3.36)=43.03$, $P<0.001$] compared to the aCSF control group (Fig. 1). These behavioural changes were not observed at the dose of 0.3 nmol/mouse. The number of closed arms entries, total number of arm entries, and percentage of time spent in closed arms and in the central platform were comparable to controls at all three doses of γ-MSHs (data not shown).

3.2. Effects of γ-MSH peptides in the elevated plus maze test on ethanol withdrawal mice

On day 12, the ethanol withdrawal control group mice showed a significant reduction of the percentage of time spent in open arms as well as the percentage of time spent in the central platform, open arm entries, and the total number of arm entries compared to the aCSF control group (Table 1). No difference was observed in the number of closed arms entries or the percentage of time spent in closed arms for ethanol control mice compared to the aCSF control group. Both γ1- and γ2-MSH-treated groups showed a much greater decrease in the percentage of open arm entries [$F(3.36)=192.08$, $P<0.001$ and $F(3.36)=193.94$, $P<0.001$] and the percentage of time spent in open arms at the doses of 1 and 2 nmol/mouse [$F(3.36)=59.88$, $P<0.001$ and $F(3.36)=61.13$, $P<0.001$] when compared to the ethanol withdrawal control group. No changes were observed in the number of closed arm entries or the percentage of time spent in closed arms in the ethanol-withdrawal and aCSF control groups. At the dose of 0.3 nmol/mouse, neither γ1- or γ2-MSH influenced ethanol withdrawal behaviour. The total number of arm entries [$F(3.36)=31.64$, $P<0.001$ and $F(3.36)=21.26$, $P<0.001$] and the percentage of time spent in the central platform were significantly reduced by both γ-MSHs at the doses of 0.3, 1 and 2 nmol/mouse only when compared to the aCSF control group.

3.3. Effects of γ-MSH peptides in acute ethanol-induced anxiolysis in the elevated plus maze test in mice

The single injection of i.p. ethanol+i.c. aCSF (control group) caused the expected anxiolytic response in mice: a significant increase in the percentage of open arm entries and the percentage of time spent in open arms compared to the aCSF control group (Fig. 2). Treatment with γ1- and γ2-MSH (1 nmol/mouse/5 μl, i.c.) induced a significant reduction of both the number of open arm entries [$F(2.27)=18.32$, $P<0.001$] and the percentage of time spent in open arms [$F(2.27)=85.17$, $P<0.001$] compared to ethanol control mice. Furthermore, ethanol-induced behavioural effects were reversed to the control levels. No changes were observed in the number of closed arm entries, the total number of arm entries and percentage of time spent in closed arms, as compared to ethanol and aCSF control groups (data not showed).

Table 1
The effects of γ1- and γ2-MSH in the elevated plus maze on anxiety-like behaviour of ethanol withdrawn mice

Treatment (i.c. and i.p. administration)	Dose (i.c. administration)	Open arm entries, %	Total arm entries, number	Time spent in open arms, %	Time spent in central platform, %
aCSF control (+saline, i.p.)	aCSF, 5 μl, i.c.	42.85±1.78	14.21±0.51	13.6±0.51	17.20±1.51
Ethanol withdrawal control (ethanol, 2 g/kg, i.p.)	aCSF, 5 μl, i.c.	35.30±1.69*	8.64±0.68*	6.78±0.54*	14.25±1.52*
γ1-MSH (+ethanol, 2 g/kg, i.p.)	0.3 3 nmol/5 μl, i.c.	33.13±1.72*	9.84±0.44*	6.73±0.45*	14.88±1.46*
γ1-MSH (+ethanol, 2 g/kg, i.p.)	1 nmol/5 μl, i.c.	5.68±0.54**++	7.74±0.63*	1.33±0.28**++	14.32±1.50*
γ1-MSH (+ethanol, 2 g/kg, i.p.)	2 nmol/5 μl, i.c.	3.25±0.49**++	7.69±0.58*	1.12±0.14**++	13.78±1.39*
γ2-MSH (+ethanol, 2 g/kg, i.p.)	0.3 nmol/5 μl, i.c.	36.99±1.84*	8.19±0.76*	6.38±0.52*	14.02±1.35*
γ2-MSH (+ethanol, 2 g/kg, i.p.)	1 nmol/5 μl, i.c.	7.03±0.61**++	7.39±0.63*	1.22±0.22**++	14.29±1.53*
γ2-MSH (+ethanol, 2 g/kg, i.p.)	2 nmol/5 μl, i.c.	3.51±0.49**++	7.40±0.90*	1.18±0.16**++	14.18±1.55*

Ethanol withdrawn mice (2 days after discontinuation of 10-day forced alcoholization) were i.c. administrated γ1- and γ2-MSH (both at the doses of 0.3, 1 and 2 nmol/mouse/5 μl) or aCSF (mouse/5 μl). After 20 min, mice were tested for anxiety-like behaviour using the elevated plus maze for 5 min.
aCSF control group animals received i.p. saline for 10 days and i.c. injections of aCSF (mouse/5 μl) on the day 12. Ethanol withdrawn control group animals received i.p. ethanol (2 g/kg) for 10 days and i.c. injection of aCSF (mouse/5 μl) on the day 12. Values are mean±SEM. $n=10$ mice/group. Significance was measured by the one-way analysis of variance (ANOVA) followed by Dunnet's post-hoc test.
*$P<0.05$; **$P<0.01$ vs. aCSF control group; +$P<0.05$; ++$P<0.01$ vs. ethanol withdrawn control group animals in ethanol-withdrawal.

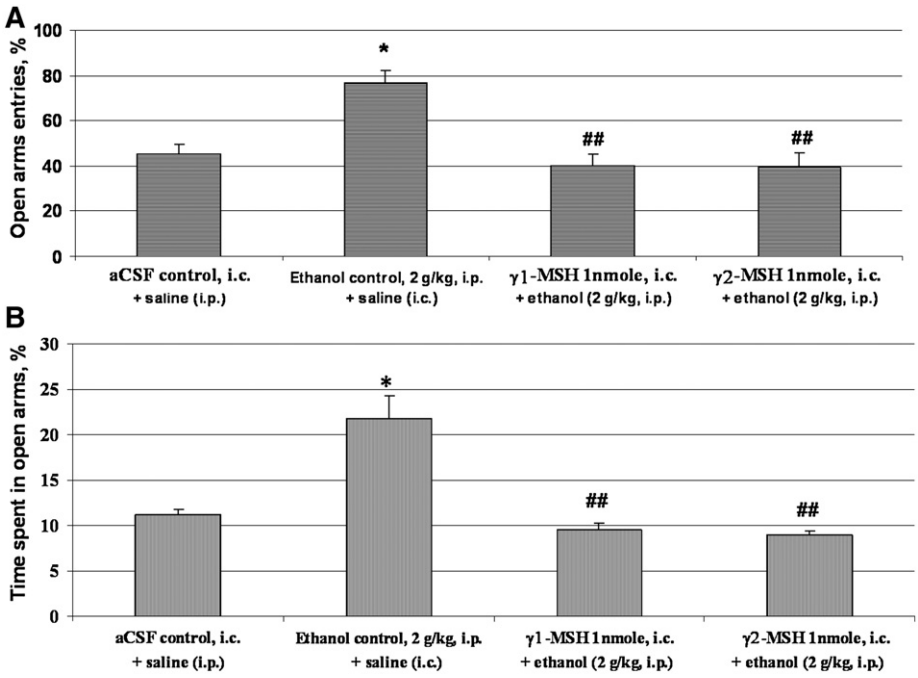

Fig. 2. Effects of γ1- and γ2-MSH (both at the dose of 1 nmol/mouse/5 μl) on the exploratory behaviour of acute ethanol-treated mice. The percentage of open arms entries (A) and percentage of time spent in open arms (B) were observed for 5 min in elevated plus maze. Ethanol (2 g/kg) was injected i.p. 45 min prior to testing and γ-MSHs or aCSF were administrated i.c. 20 min before the elevated plus maze test. Values are mean ± SEM. $n = 10$ mice/group. Significance was measured by the one-way analysis of variance (ANOVA) followed by Dunnet's post-hoc test. *$P < 0.05$ vs. aCSF control group; ##$P < 0.01$ vs. ethanol control group.

3.4. Cataleptic activity

The evaluation of cataleptic activity immediately after the plus maze test showed the absence of catalepsy in all experiments.

4. Discussion

The aim of the present study was to clarify the function of γ1- and γ2-MSH and their role in anxiogenic or anxiolytic effects in the elevated plus maze test, as well as their influence of anxiogenic responses caused by ethanol withdrawal or influence of the acute ethanol-induced anxiolytic responses. To our knowledge, this is the first study to compare the action of different members of the MSH peptide family on anxiety. Our data are comparable to the anxiolytic actions of α-MSH recently described (Gonzalez et al., 1996; Vecsernyes et al., 2000; Kokare et al., 2006).

In the present study, i.c. injections in mice were chosen because we have previously used the same route of γ1- and γ2-MSH administration to examine the influence of these peptides on ethanol-induced analgesia (Klusa et al., 2001). Our findings demonstrate that both γ1- and γ2-MSH show anxiogenic effects, as tested by the elevated plus maze test. At the doses of 1 and 2 nmol/mouse both γ-MSHs significantly reduced the percentage of open arm entries and the percentage of total time spent in open arms. The lowest dose of 0.3 nmol/mouse did not influence behaviour compared to aCSF control animals. To establish that this was truly an anxiogenic effect and not a response attributable to cataleptic action, we assessed catalepsy in the mice immediately after completing the elevated plus maze test. The data clearly showed the absence of catalepsy, thus confirming the anxiogenic effect of γ-MSHs. There is considerable similarity between the anxiogenic effects of γ-MSHs shown here and that of α-MSH previously demonstrated (Gonzalez et al., 1996; Vecsernyes et al., 2000; Kokare et al., 2006). Although the present experiments were carried out in mice, the doses that caused anxiogenic behaviours are comparable to those used in previous studies. We used 1 and 2 nmol i.c. in mice while others used 1–5 mg intracerebroventricularly in rats (Kokare et al., 2006), which corresponds to 0.6–3.2 nmol/animal. Our present data and those obtained by other authors therefore indicate that α- and γ-MSHs can be attributed to the endogenous anxiogenic substances and suggest their involvement in the development of anxiety disorders.

Similar to α-MSH action (Kokare et al., 2006), γ-MSHs also reduced the anxiolytic effect induced by ethanol. In this experiment, we tested only one dose (1 nmol/mouse) that gave a stable anxiogenic effect. It is interesting that both γ-MSHs (at the doses of 1 and 2 nmol/mouse, but not 0.3 nmol/mouse) enhanced the anxiogenic behaviour caused by ethanol withdrawal. Both doses reduced the percentage of open arm entries and the percentage of time spent in open arms (compared to ethanol control group), indicating that γ-MSHs act in an additive or synergistic manner to ethanol withdrawal.

Based on the present findings, we cannot propose a precise mechanism that would explain why γ-MSHs *per se* cause an anxiogenic effect and augment the ethanol withdrawal-induced anxiogenic responses in mice. It is possible to associate the anxiogenic effect of γ-MSHs with their binding to melanocortin receptors, particularly the MC3R and MC4R subtypes, since these receptors interact with γ-MSHs with high affinity (Wikberg, 1999; Wikberg et al., 2000; Schioth, 2001). The role of MC4R in the anxiogenic effect of α-MSH has been previously described. The MC3/MC4 receptor antagonist HS014 has been shown to inhibit the anxiogenic effect caused by ethanol withdrawal, and HS014 coupled with antiserum to α-MSH has been shown to enhance the anxiolytic effect of ethanol (Kokare et al., 2006). These data allow the speculation of common mechanisms that might explain the anxiogenic effects of γ-MSHs and α-MSH as well as the action of MSHs on ethanol. Many studies have attempted to identify the mechanisms of anxiety, although the melanocortinergic impact on anxiogenic development is a comparatively recent finding (Chaki et al., 2003; Kokare et al., 2006). Interestingly, increased proopiomelanocortin mRNA levels have been found in the mediobasal hypothalamus in ethanol-withdrawn rats (Rasmussen et al., 2002), while other data has shown that ethanol may inhibit the melanocortin system (Rainero et al., 1990; Rasmussen et al., 2000, 2001; Navarro et al., 2005).

Ethanol withdrawal-induced anxiety, however, also involves different non-melanocortinergic mechanisms, such as those mediated by GABA-A and GABA-B receptors (File et al., 1991), CRF receptors (Dave et al., 1986;

Overstreet et al., 2005), and 5-HT1A receptor (Lal et al., 1991). These receptor subtypes have been shown to be essential components of ethanol action. The use of diazepam, a GABA receptor ligand, is well established as an effective treatment for ethanol withdrawal. In this context, it is interesting to note that the α-MSH anxiogenic effect in the elevated plus maze test in rats is attenuated by diazepam as well as muscimol, findings that suggest the involvement of the GABAergic system in reversing the anxiogenic-like effect of α-MSH (Rao et al., 2003). The importance of non-melanocortinergic mechanisms in the γ-MSHs-induced central response, such as GABA- and dopaminergic effects, has been demonstrated previously (Klusa et al., 2001; Jansone et al., 2004). We showed that γ1-MSH (injected i.c. in mice) significantly attenuated analgesia induced by ethanol and diazepam, and that α-MSH reduced analgesia induced by diazepam but not by ethanol (Klusa et al., 2001).

The binding of γ-MSH peptides to the GABA-A receptor GABA site, however, showed very low affinity for γ1-MSH (Ki value = 290 ± 78 μM) and no binding at all for γ2-MSH. This finding suggests that γ-MSHs behavioural responses are mostly due to GABAergic modulation (Dzirkale et al., 2005).

The most attractive explanation for the idea of a common molecular mechanism for the action of MSH peptides and ethanol may be found in their common cellular signalling pathways, such as adenylyl cyclase (AC) activation and secondary messenger cAMP-induced cascades. It is well documented that MSH peptides stimulate the activity of AC, resulting in the accumulation of cAMP (Wikberg, 1999). Recently, ACs were described as a new class of alcohol-responsive proteins (Yoshimura et al., 2006). Moreover, CNS responses to ethanol have been attributed to the involvement of cAMP-protein kinases-, PKA-, and cAMP response element (CRE)-mediated cascades (Asyyed et al., 2006). Taking these molecular mechanisms into account, we suggest that the potentiating effect of γ-MSH peptides on anxiogenic responses caused by ethanol withdrawal are best accounted for by cellular effects common to ethanol and γ-MSHs. The details of such a mechanism remain to be elucidated.

In conclusion, the central anxiogenic responses of γ1- and γ2-MSH demonstrated in the present study show for the first time very close similarity to the anxiogenic effect of α-MSH reported previously by other authors. Both α-MSH (Kokare et al., 2006) and γ-MSHs were capable of attenuating the ethanol anxiolytic effect. Moreover, both γ-MSH peptides enhanced the ethanol withdrawal-induced anxiogenic action in mice. These data suggest that γ1- and γ2-MSH, together with α-MSH (and perhaps b-MSH), belong to an anxiogenic peptide family that plays an important role in anxiety disorders. Moreover, these peptides could be involved in regulating the development of alcohol dependence and/or alcohol withdrawal-induced behavioural events. Further studies are needed to elucidate the impact of the MSH peptide family on ethanol-induced disturbances in the CNS and the possibility of treating them by selective cellular targeting.

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References

Allan AM, Burnett D, Harris A. Ethanol-induced changes in chloride flux are mediated by both $GABA_A$ and $GABA_B$ receptors. Alcoholism. Clin Exp Res 2008;22:3–9.
Aguayo LG, Peoples RW, Yeh HH, Yevenes GE. GABA(A) receptors as molecular sites of ethanol action. Direct or indirect actions? Curr Top Med Chem 2002;8:869–85.
Asyyed A, Storm D, Diamond I. Ethanol activates cAMP response element-mediated gene expression in select regions of the mouse brain. Brain Res 2006;1106:63–71.
Chaki S, Ogawa S, Toda Y, Funakoshi T, Okuyama S. Involvement of the melanocortin MC4 receptor in stress-related behavior in rodents. Eur J Pharmacol 2003;474:95-101.
Cole JC, Littleton JM, Little HJ. Acamprosate, but not naltrexone, inhibits conditioned abstinence behaviour associated with repeated ethanol administration and exposure to a plus-maze. Psychopharmacology 2000;147:403–11.
Dave JR, Eiden LE, Karanian JW, Eskay RL. Ethanol exposure decreases pituitary corticotropin-releasing factor binding, adenylate cyclase activity, proopiomelanocortin biosynthesis, and plasma beta-endorphin levels in the rat. Endocrinology 1986;118:280–6.
Doremus TL, Brunell SC, Varlinskaya EI, Spear LP. Anxiogenic effects during withdrawal from acute ethanol in adolescent and adult rats. Pharmacol Biochem Behav 2003;75:411–8.
Dzirkale Z, Rumaks J, Dilendorfa J, Jurgaitite E, Jansone B, Svirskis S, et al. Diversity of melanocortin-induced behavioral responses, analgesic mechanisms and receptor-binding characteristics. European Neuropeptide Club 15th Conference; 2005. p. 71. Abstract Book.
Faingold CL, N'Gouemo P, Riaz A. Ethanol and neurotransmitter interactions—from molecular to integrative effects. Review Prog Neurobiol 1998;5:509–35.
File SE, Zharkovsky A, Gulati K. Effects of baclofen and nitrendipine on ethanol withdrawal responses in the rat. Neuropharmacology 1991;30:183–90.
Gonzalez LE, Andrews N, File SE. 5-HT1A and benzodiazepine receptors in the basolateral amygdala modulate anxiety in the social interaction test, but not in the elevated plus-maze. Brain Res 1996;732:145–53.
Jansone B, Bergstrom L, Svirskis S, Lindblom J, Klusa V, Wikberg JES. Opposite effects of gamma(1)- and gamma(2)-melanocyte stimulating hormone on regulation of the dopaminergic mesolimbic system in rats. Neurosci Lett 2004;361:68–71.
Kameda SR, Frussa-Filho R, Carvalho RC, Takatsu-Coleman AL, Ricardo VP, Patti CL, et al. Dissociation of the effects of ethanol on memory, anxiety, and motor behavior in mice tested in the plus-maze discriminative avoidance task. Psychopharmacology (Berl) 2007;192:39–48.
Klusa V, Svirskis S, Opmane B, Muceniece R, Wikberg JES. Behavioural responses of γ-MSH peptides administered into the rat ventral tegmental area. Acta Physiol Scand 1999;67:99-104.
Klusa V, Germane S, Svirskis S, Opmane B, Wikberg JES. The γ2-MSH peptide mediates a central analgesic effect via a GABA-ergic mechanism that is independent from activation of melanocortin receptors. Neuropeptides 2001;35:50–7.
Kobayashi T, Araki T, Itoyama Y, Takeshita M, Ohta T, Oshima Y. Effects of L-DOPA and bromocriptine on haloperidol-induced motor deficits in mice. Life Sci 1997;61:2529–38.
Kokare DM, Chopde CT, Subhedar NK. Participation of a-melanocyte stimulating hormone in ethanol-induced anxiolysis and withdrawal anxiety in rats. Neuropharmacology 2006;51:536–45.
Lal H, Prather PL, Rezazadeh SM. Anxiogenic behavior in rats during acute and protracted ethanol withdrawal: reversal by buspirone. Alcohol 1991;8:467–71.
Lindblom J, Schioth HB, Larsson A, Wikberg JES, Bergstrom L. Autoradiographic discrimination of melanocortin receptors indicates that the MC3 subtype dominates in the medial rat brain. Brain Res 1998;10:161–71.
Naftolin F, Shanabrough M, Leranth C. Pro-opiomelanocortin (POMC) neurons in the mediobasal hypothalamus (MBH) projecting to the medial preoptic area (MPO) are synaptic targets of GABAergic terminals. Society for Neuroscience Abstracts 1990;393:1.
Navarro M, Cubero I, Chen AS, Chen HY, Knapp DJ, Breese GR, et al. Effects of melanocortin receptor activation and blockade on ethanol intake: a possible role for the melanocortin-4 receptor. Alcohol Clin Exp Res 2005;29:949–57.
Overstreet DH, Knapp DJ, Breese GR. Pharmacological modulation of repeated ethanol withdrawal-induced anxiety-like behavior differs in alcohol-preferring P and Sprague–Dawley rats. Pharmacol Biochem Behav 2005;81:122–30.
Pellow S, File SE. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. Pharmacol Biochem Behav 1986;24:525–9.
Rainero I, De Gennaro T, Visentin G, Brunetti E, Cerrato P, Torre E, et al. Effects of chronic ethanol treatment on alpha-MSH concentrations in rat brain and pituitary. Neuropeptides 1990;15:139–41.
Rao TL, Kokare DM, Sarkar S, Khisti RT, Chopde CT, Subhedar N. GABAergic agents prevent alpha-melanocyte stimulating hormone induced anxiety and anorexia in rats. Pharmacol Biochem Behav 2003;76:417–23.
Rasmussen DD, Boldt BM, Bryant CA, Mitton DR, Larsen SA, Wilkinson CW. Chronic daily ethanol and withdrawal: 1. long-term changes in the hypothalamo-pituitary–adrenal axis. Alcohol Clin Exp Res 2000;24:1836–49.
Rasmussen DD, Mitton DR, Green J, Puchalski S. Chronic daily ethanol and withdrawal: 2. behavioral changes during prolonged abstinence. Alcohol Clin Exp Res 2001;25:999-1005.
Rasmussen DD, Boldt BM, Wilkinson CW, Mitton DR. Chronic daily ethanol and withdrawal: 3. forebrain pro-opiomelanocortin gene expression and implications for dependence, relapse, and deprivation effect. Alcohol Clin Exp Res 2002;26:535–46.
Roselli-Rehfuss L, Mountjoy KG, Robbins LS, Mortrud MT, Low MJ, Tatro JB, et al. Identification of a receptor for gamma melanotropin and other proopiomelanocortin peptides in the hypothalamus and limbic system. Proc Natl Acad Sci USA 1993;90:8856–60.
Schioth HB. The physiological role of melanocortin receptors. Vitam Horm 2001;63:195–232.
Schioth HB, Muceniece R, Wikberg JES, Chhajlani V. Characterization of melanocortin receptor subtypes by radioligand binding analysis. Eur J Pharmacol 1995;288:311–7.
Spanagel R, Heilig M. Addiction and its brain science. Addiction 2005;100:1813–22.
Takagi H, Shiomi M, Ueda H, Amano H. Morphine-like analgesia by a new dipeptide, l-tyrosyl-l-arginine (Kyotorphin) and its analogue. Eur J Pharmacol 1979;55:109–11.
Vecsernyes M, Biro E, Gardi J, Julesz J, Telegdy G. Involvement of endogenous corticotropin-releasing factor in mediation of neuroendocrine and behavioral effects to alpha-melanocyte-stimulating hormone. Endocr Res 2000;26:347–56.
Wikberg JES. Melanocortin receptors: perspectives for novel drugs. Eur J Pharmacol Rev 1999;375:295–310.
Wikberg JES, Muceniece R, Mandrika I, Prusis P, Lindblom J, Post C, et al. New aspects on the melanocortins and their receptors. Pharmacol Res 2000;42:393–420.
Xi ZX, Stein E. Nucleus accumbens dopamine release modulation by mesolimbic $GABA_A$ receptors: an in vivo electrochemical study. Brain res 1998;798:156–65.
Yoshimura M, Pearson S, Kadota Y, Gonzalez CE. Identification of ethanol responsive domains of adenylyl cyclase. Alcohol Clin Exp Res 2006;30:1824–32.