Effects of melanotan II, a melanocortin agonist, on grooming and exploration in rats after repeated restraint/immobilization

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Abstract

2 mg/kg melanotan II (MTII, administered i.p.), a cyclic peptide analog of α-melanocyte stimulating hormone, at a single dose increased grooming in naive rats placed in an unfamiliar open-field device without changing locomotion or rearing. Male rats exposed to restraint/immobilization stress (IS) for 1 h on three consecutive days displayed increased grooming after the second stressor exposure, compared to pre-stress levels. MTII, administered to the rats after IS, enhanced the grooming response compared both to the pre- and post-stress values. The increase was greatest after the first dose and declined over the following two applications. As to the locomotion of rats in the entire experimental space, IS reduced the distance moved only after the first two stressor exposures; MTII did not influence these alterations. Locomotion in the central part of arena was not reduced by the stressor or by MTII, on the contrary, there was an increase in both groups after the third intervention. The only observed change in rearing was an increase in the MTII group after the third restraint exposure. Thus, MTII selectively increased grooming without markedly affecting the spatio-temporal structure of locomotor behavior in the open-field. The decline of MTII enhanced grooming over the three test days may be interpreted in terms of adaptation to the stressor and of the developing tolerance to the peptide.

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Brain melanocortin α-melanocyte stimulating hormone (α-MSH) and adrenocorticotrophic hormone (ACTH) modulate a variety of neurophysiological phenomena including stress-induced neurochemical and behavioral responses and disorders in laboratory rodents [1,6,7]. The demonstration of brain specific melanocortin receptor subtypes expressed in rat brain indicates that central actions of α-MSH are mediated primarily by melanocortin MC3 and MC4 receptors [1,2]. Rat grooming behaviors were among the first described behaviors elicited either by mild stress or by intracerebral injection of melanocortin receptor agonists α-MSH and ACTH [6,11,12]. Recently it has been shown that melanotan II (MTII), a more selective melanocortin MC4 receptor agonist, dose-dependently increased grooming episodes observed in rats placed in a novel environment [1]. The role of melanocortins in modulation of grooming behavior in rats is further supported by the finding that SHU919, an analogue acting as an inhibitor on melanocortin receptors, inhibited both novelty and α-MSH- or MTII-induced grooming [1].

Another role melanocortins play in stress-induced behavioral responses is the induction of anxiety [4,10]. More recently, i.c.v. administration of α-MSH and as well as MTII dose-dependently reduced the number of licking periods in the rat Vogel conflict test, which suggested that stimulation of melanocortin MC4 receptor causes anxiogenic-like activity [2]. In support for this view, melanocortin MC4 selective antagonist MCL0020 given i.c.v. prevented swim stress-induced reduction in the mouse light–dark exploration test [2]. These findings show that melanocortin MC4 receptor might be actually related to at least some stress-induced alterations in behavioral responses.

In previous studies we showed that restraint/immobilization one hour in duration exerts both short- and long-term effects on spontaneous behavior of rats in the open-field test [16,17]. The aim of the present study was to evaluate if and to what extent MTII can modulate effects of restraint stress on spontaneous behavior of rats including grooming pattern. To explore possible anxiogenic-like action of MTII, we recorded locomotor activity of rats close to the wall and in the central part of the arena, a
reduction of the latter being commonly considered as an index of anxiety [18,19,21].

Animals: Male Wistar rats (Velaz, Czech Republic), with starting body weights of 290–305 g, were used. Daily handled rats (during daily weighing animals were kept in the hand for about 1 min) had free access to a standard pellet food (ST1, Velaz, Czech Republic) and water. Rats were housed in a room maintained on a 12-h light/dark cycle (lights on 6–18) and temperature (21 ± 1 °C). Four rats were housed per cage (42 cm × 26 cm). Treatment of animals was in accordance with the Declaration of Helsinki Guiding Principles on Care and Use of Animals (DHEW Publication, NHI 80-23).

Drug: Melanotan II, a cyclic peptide analog of α-MSH, was dissolved in saline and injected i.p. in a dose of 2 mg per 2 ml/kg b.w. Control animals received the same volume of saline.

Stress procedure: Rats were exposed to 60 min restraint/immobilization (IS) applied by fixing front and hind legs of the rat with adhesive plaster; then the animal was restrained in a snug-fitting plastic-mesh. This mesh was bent to conform to the size of the individual animal and a bandage fixed this shape of mesh. During the stress the animals were kept in a vertical position [16,17]. After the exposure to the stressor, the animals were returned to the home cage. For the stress procedure the rats were transferred to a separate room. Two different persons performed the stress procedure and the behavioral testing.

Behavioral testing: Rats were randomly assigned to two groups in both experiments. Behavior of rats was video-monitored by an automated activity monitoring system (AnyMaze, Stoelting, U.S.A.) in a circular arena with the diameter of 150 cm; the walls were 50 cm high. While the movement distance and the total movement distance in the inner zone (the diameter of 110 cm) were recorded automatically, the number of rearing and the total time spent in grooming were measured by an experimenter. We did not differentiate between grooming elements (face washing, licking the body fur, genital area, limbs and tail, and scratching). Behavioral testing began 60 min after the termination of stress and/or injection (saline or MTII). Data were collected for 10 min (Experiment 1) or 5 min (Experiment 2) period. Both experiments were performed from 8 a.m. to 1 p.m. in a separate room illuminated by a fluorescent light located on the ceiling.

Statistics: The Systat 10 software (SPSS, Inc., Chicago, U.S.A.) was used to evaluate behavioral data. In the second experiment, a two-way analysis of variance (ANOVA) with factors of treatment and day was followed with the Bonferroni adjusted post hoc tests to compare individual days within both the IS + saline (control) and IS + MTII (experimental) groups. To compare the data within a particular day between both groups the t-test was used in both experiments. Data were expressed as mean ± S.E. Statistical significance was accepted when p ≤ 0.05.

Experiment 1: MTII-treated animals spent a significantly longer time (s) in grooming than the controls (42.4 ± 10.1, n = 8 and 10.6 ± 3.2, n = 8, respectively; t = 3.0, p = 0.01). No difference between both groups was found in the total distance moved and the number of rearing.

Experiment 2: On Day 1, all animals were tested in the open-field device without any intervention. On Day 2, half of animals (n = 9) received IS + saline (control group) and the other half of animals (n = 9) received IS + MTII (experimental group). The same procedure was repeated on Day 3 and Day 4.

The results are presented in Fig. 1. A two-way ANOVA on the total time spent in grooming revealed a significant effect of treatment (F(1,64) = 30.18, p < 0.0001), a significant effect of day (F(3,64) = 11.68, p < 0.0001), and treatment × day interaction (F(3,64) = 6.26, p = 0.0009). Animals given IS + MTII devoted significantly more time to the grooming on Day 2, 3 and 4 as compared with IS + saline group. No change in grooming time
was observed in the IS + saline-treated animals throughout the experiment. In IS + MTII-treated animals the highest grooming level was found on Day 2, then it gradually decreased; nevertheless, on Day 4 the level was significantly increased when compared with Day 1.

A two-way ANOVA on the total distance moved revealed a significant effect of day \( F(3,64) = 5.95, p = 0.0018 \), but no effect of treatment \( F(1,64) = 0.047, p = 0.83 \) and no significant interaction between treatment and day \( F(3,64) = 0.099, p = 0.96 \). In both groups there was a significant decrease of the total movement distance on Day 2. On Day 4 the movement level returned to that exhibited on Day 1.

A two-way ANOVA on the distance moved in the inner zone revealed a significant effect of day \( F(3,64) = 4.42, p = 0.007 \), but no effect of treatment \( F(1,64) = 0.26, p = 0.61 \) and no effect of interaction \( F(3,64) = 0.29, p = 0.83 \). On Day 4, only animals given IS + MTII exhibited significantly increased movement in the inner zone.

Finally, as to the number of rearing, a two-way ANOVA revealed a significant effect of treatment \( F(1,64) = 4.14, p = 0.046 \), a significant effect of day \( F(3,64) = 5.80, p < 0.01 \), but no significant interaction between treatment and day \( F(3,64) = 0.79, p = 0.50 \). There was no change across days in the control group. On the contrary, the rearing level in animals given IS + MTII increased significantly on Day 4, but did not reach a significant difference when compared to the controls.

**Discussion:** The results revealed increased grooming after MTII given intraperitoneally. In Experiment 1, a single dose of MTII increased the amount of grooming in rats exposed to a novel environment. The main finding in Experiment 2 (Fig. 1), is the high enhancement of grooming activity induced by combination of IS stressor exposure and MTII treatment. The most striking increase appeared on the first day of the combined treatment (Day 2), compared to grooming amount elicited by IS presentation only. Several types of stressors-like placement in the novel environment, handling, water immersion, noise or social interaction were found to elicit grooming [3,9,12]. Repeated restraint stress has also been reported to increase grooming [5], however, the restraint inducing severe stress reduced grooming response [12]. Stress of electrical foot shock has been shown to suppress or to stimulate grooming depending on the strength or number of shocks [13–15]. In the present study IS did not increase grooming to a significant degree; however, the effects of combination of MTII and IS suggest that the duration and type of stress used induce neurochemical and endocrine changes favorable for grooming behavior to occur. In several investigations novelty-induced grooming habituated with repeated testing [1,12], however, constant response over seven exposures has been reported as well [15]. In the present study the enhanced grooming response after MTII injections declined over repeated sessions. In this connection it is noteworthy that repeated injections of ACTH fragment have been shown to produce comparable amounts of grooming if separated by at least 24 h; if shorter intervals between injections were employed, rapid tolerance developed [3,12]. We assume that the decline of the grooming response after repeated MTII treatment may be related to the developing adaptation to the stressor exposure.

The reduction of locomotion observed after the first IS exposure only (Day 2) also points to the process of habituation to the stressor effects. The recovery of reduced locomotion after the second and/or third stressor exposure was also found in previous study obtained under identical conditions [16]. In support of the participation of adaptation process also vertical exploratory activity – rearing – increased on Day 4.

In a novel environment rodents prefer to walk close to the walls. Locomotion and time spent in the inner zone of the arena are considered as a variable reflecting the anxiety level [8,19,20]. In our experimental approach, i.e. using a large size open-field, we found no reduction in the central locomotion after IS. On the contrary, MTII + IS-treated rats exhibited higher inner zone locomotor activity on Day 4. This is in contrast with findings that MTII exerts an anxiogenic-like effect after i.c.v. administration in the test of punished drinking; a melanocortin MC4 receptor antagonist prevented swim stress-induced behavioral alteration in the mouse light–dark exploration test [2]. Both methods may be considered as stronger stressogenic events than IS. We also observed that timing of stress and testing influence the resulting behavioral performance in rats [16]. Lowered level of emotionality induced by handling could also be involved in the observed absence of movement depression in the inner zone.

Grooming behavior induced by MTII was potentiated in IS stressed rats. In our view, it is conceivable to assume that this increase was caused by a synergic action of MTII and stress activators of MC4 brain receptors. This notion is supported by the finding of gradual decline of grooming after repeated MTII treatment that can be explained in terms of adaptation or habituation reflected in the recovery of locomotion and rearing in IS stressed rats. This behavioral change indicates lesser stimulation of hypothalamic/hypophyseal/adrenal axis. Also, in preliminary experiment we observed an indication of developing tolerance to the MTII effects.

In conclusion, if we accept that grooming is elicited by stimulation of MC4 receptors, then the present results suggest that MTII, given also intraperitoneally, penetrates the blood–brain barrier to such an extent that its brain concentrations produce measurable behavioral effects.

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**References**


