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Short communication

The potent melanocortin receptor agonist melanotan-II promotes peripheral nerve regeneration and has neuroprotective properties in the rat

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Abstract

The neurotrophic and neuroprotective potential of the α-melanocyte-stimulating hormone (α-MSH) analog cyclo-[Ac-Nle^4,Asp^5, D-Phe^7,Lys^{10}]α-MSH-(4–10) amide (melanotan-II), a potent melanocortin receptor agonist, was investigated. The sciatic nerve crush model was used as a paradigm to investigate the neurotrophic properties of melanotan-II. Melanotan-II significantly enhanced the recovery of sensory function following a crush lesion of the sciatic nerve in the rat at a dose of 20 μg kg^{-1} per 48 h, s.c., but not at a dose of 2 or 50 μg kg^{-1}. In addition, we observed that melanotan-II also possesses neuroprotective properties, as it partially protected the nerve from a toxic neuropathy induced by cisplatin. Thus, the present data for the first time demonstrate the effectiveness of the potent α-MSH analog melanotan-II in nerve regeneration and neuroprotection.

Keywords: Melanocortin receptor; α-MSH (α-melanocyte-stimulating hormone); Nerve crush; Cisplatin-induced neuropathy; Melanotan-II

1. Introduction

Over the years, numerous studies have shown the importance of melanocortins in the development and maintenance of the nervous system (Strand et al., 1993). Melanocortin peptides are neuropeptides derived from the precursor molecule pro-opiomelanocortin (POMC) by tissue-specific splicing; they include adrenocorticotropic hormone (ACTH) and α-melanocyte-stimulating hormone (α-MSH).

Several of the melanocortins, including α-MSH, have been shown to improve recovery of function after peripheral nerve damage (for a review, see Strand et al., 1993), as well as after experimental spinal cord injury (Lankhorst et al., 1999). Histological examination of injured nerve after α-MSH treatment has revealed a marked effect on the number of neurofilament positive fibers in the regenerating nerve, indicating that α-MSH increases the number of sprouts formed after damage (Joosten et al., 1999). In addition, results of in vitro studies have demonstrated that α-MSH increases the number and the length of neurites formed in cultured dorsal root ganglion neurons (Haynes and Semenenko, 1989). Although these neurotrophic effects of α-MSH and other melanocortins are well established, the mechanisms underlying these effects remain, as yet, largely unknown.

Adan et al. (1996) demonstrated that α-MSH promotes neurite-like outgrowth in neuro 2A cells through a melanocortin receptor. Neuro 2A cells express several types of melanocortin receptors, but the outgrowth-promoting effect of α-MSH could be blocked with a specific melanocortin MC_4 receptor antagonist ([D-Arg]ACTH-(4–10)), suggesting that the melanocortin MC_4 receptor is involved in this effect of α-MSH. These findings have been confirmed in a subsequent similar study by Davis et al. (1999).

In the present study, we intended to further explore the possible beneficial effects of cyclo-[Ac-Nle^4,Asp^5,D-Phe^7, Lys^{10}]α-MSH-(4–10) amide (melanotan-II) in peripheral nerve regeneration and neuroprotection. Whereas specific melanocortin receptor antagonists are available, specific melanocortin receptor agonists are not. We, therefore, decided to investigate the effects of the most potent melanocortin receptor ligand, i.e., the cyclic α-MSH-related peptide melanotan-II in sciatic nerve crush and cisplatin-induced neuropathy. Previously, this cyclic ligand was shown to have a

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high affinity for the melanocortin MC_1 and MC_4 receptors, with a lower affinity for the melanocortin MC_3 and MC_5 receptors (see Wikberg, 1999), and to activate the brain melanocortin MC_4 receptor in vivo (Adan et al., 1999).

In the present study, we show a dose-dependent facilitation of sensorimotor recovery following a sciatic nerve crush and a protection against cisplatin-induced neuropathy by melanotan-II.

2. Materials and methods

2.1. Animals and group sizes

All experiments were performed in accordance with the guidelines of the Committee on the Use of Experimental Animals of Utrecht University. In all experiments, male Wistar rats (U:WU:CPB) were used. The rats were allowed to acclimatize for a week after arrival in our animal facilities. They were housed on sawdust bedding in groups of two in type 3 Macrolon cages. Food (commercial rat chow, Hope Farms, Woerden, The Netherlands) and water were available ad libitum. A 12:12-h light/dark regime was employed, with lights on at 7:30 a.m.

In the *MT-II/sciatic nerve crush* experiment, 48 rats (130 ± 10 g) were used. The rats were assigned at random to 1 of 4 groups of 12 rats and treated (s.c.) with saline (0.5 ml/48 h s.c.) or melanotan-II at a dose of 2, 20 or 50 μg kg^{-1}, respectively. The peptide was freshly dissolved in saline and administered subcutaneously every 48 h. One rat was excluded from the study due to excessive autotomy (in the 2.0 μg kg^{-1} melanotan-II group). There was no difference in mean body weight between the groups at any point in the study (data not shown).

In the *MT-II/cisplatin-induced neuropathy* experiment, 48 rats (275 ± 25 g) were randomly divided over 4 groups. The rats of one group ($n = 12$) served as age-matched controls and received no cisplatin. The 48 remaining animals were treated with cisplatin and received (s.c.) co-treatment with either saline (0.5 ml, $n = 12$), 2 μg kg^{-1} melanotan-II ($n = 12$) or 20 μg kg^{-1} melanotan-II ($n = 12$), administered subcutaneously every 48 h.

2.2. Sciatic nerve lesion and measurements

2.2.1. Surgery

The rats were anaesthetized with a subcutaneous injection of Hypnorm® (Janssen Pharmaceutical, Grove, Oxford) at a dose of 0.8 ml kg^{-1} body weight. The sciatic nerve was crushed following a standard protocol (see Ter Laak et al., 2000a). Briefly, a small incision was made from the right hip (trochanter major) toward the knee joint. The sciatic nerve was carefully exposed and crushed with hemostatic forceps just distal of the point where it surfaces from under the gluteus maximus muscle. The forceps were closed around the nerve and held for 30 s.

2.2.2. Sensory recovery

Sensory recovery was monitored with the foot reflex withdrawal test (see Ter Laak et al., 2000a). The foot reflex withdrawal test was first performed at day 4 to confirm the effectiveness of the surgery. From day 12 onward, the foot reflex withdrawal test was repeated every 24 h to monitor the return of sensory function. Briefly, the rat is immobilized by hand and the plantar side of the foot of the injured paw is electrically stimulated with a small electrical current varying from 0.1 to 0.6 mA. A rat with intact innervation of the foot sole will retract its foot as soon as it contacts the poles of the stimulator at the lowest setting. Rats recently subjected to a sciatic nerve crush do not retract their foot even when the maximal stimulus (0.6 mA) is applied. However, with the return of sensory function, the rat will react to electrical stimulation by withdrawing its foot at decreasing currents. The rat is considered to be fully recovered (100%) from its nerve injury when it reacts to a stimulus of 0.1 mA.

2.3. Cisplatin-induced neuropathy

Cisplatin neuropathy was induced using a standard protocol as previously described (see Ter Laak et al., 2000a). In short, cisplatin was administered twice a week at a dose of 2 mg kg^{-1} by intraperitoneal injection. Routinely, rats thus treated develop a sensory neuropathy after 2–3 weeks, as evidenced by slowing of the sensory nerve conduction velocity. Rats were anesthetized with Hypnorm® (Janssen Pharmaceutical) at a dose of 0.3 ml kg^{-1} body weight (s.c.). Sensory nerve conduction velocity was measured as follows: sciatic and tibial nerves were stimulated both at the sciatic notch and at the ankle by means of monopolar needle electrodes. The anode is placed 5 mm proximal to the cathode. Upon stimulation of these mixed peripheral nerves, two responses can be recorded from the small muscles of the foot by means of surface electrodes: First, the M-response due to direct stimulation of α-motor neurons. Second, the H-response due to stimulation of the afferent I^A-fibers, which monosynaptically excite α-motor neurons in the spinal cord. The H-reflex-related sensory nerve conduction velocity can then be calculated from the latencies of the response and the distance between the two stimulation points.

2.4. Drugs

Melanotan-II was obtained from Bachem (Feinchemicalien, Bubendorf, Switzerland). Cisplatin solution (0.5 mg ml^{-1} Platosin®, Pharmachemie, Haarlem, The Netherlands) was diluted with saline to a final concentration of 0.05 mg ml^{-1}. The solution was freshly prepared before use.

2.5. Statistics

All statistical analyses were performed with the statistical software package SPSS 8.0. Body weights, percentage of sensory recovery after nerve crush and sensory nerve con-

duction velocity data were assessed with an analysis of variance (ANOVA) for repeated measurements followed by a Bonferroni post-hoc test. Data are presented as means ± standard errors of the mean. Treatment of the animals was blind to the investigators. Codes were only broken after the data had been analyzed.

3. Results

3.1. Melanotan-II facilitates sensory recovery from mechanical injury of the sciatic nerve

In Fig. 1, the data are expressed as the mean percentage of recovery over days. All melanotan-II-treated groups were consistently better in the recovery of sensory function as compared to the saline group. However, only the effect of the 20 μg kg^{-1} melanotan-II dose reached significance (post-operative days 17–26, ANOVA for repeated measurements, $P = 0.02$).

3.2. Melanotan-II ameliorates cisplatin-induced neuropathy

3.2.1. Cisplatin and body weight

The body weight steadily increased in the age-matched controls. The cisplatin-treated groups had significantly lower body weights as compared to those of the age-matched controls from week 2 onward (a cumulative dose of cisplatin of 8 mg kg^{-1}). There was no difference in body weight between the cisplatin groups (data not shown).

3.2.2. Sensory nerve conduction velocities

At the onset of the experiment (see Fig. 2), before cisplatin treatment started, the sensory nerve conduction velocity was approximately 54 m s^{-1} in all groups (week 0).

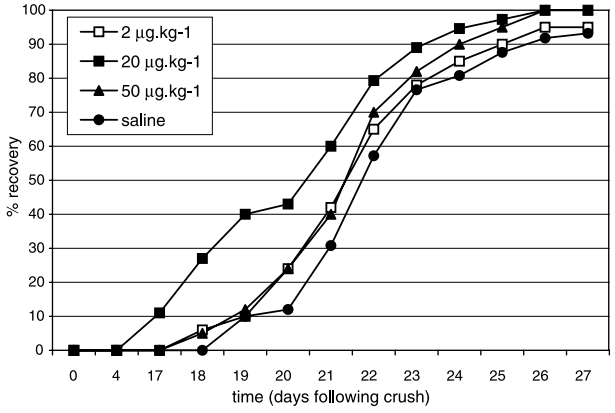

Fig. 1. The data for all treatment groups are presented as mean percentage of recovery per day. Melanotan-II in a dose of 20 μg kg^{-1} reached significance over a total period of 9 days (post-operative days 17–26, $P = 0.02$, ANOVA for repeated measurements). The other dosages of melanotan-II yielded no significant effects, but did show consistently higher mean percentages of recovery than the saline-treated rats.

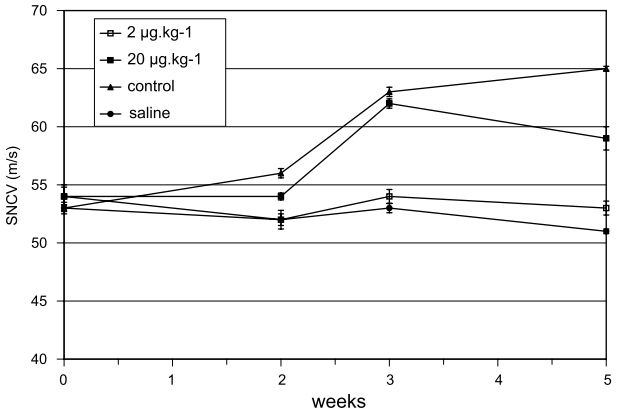

Fig. 2. Sensory nerve conduction velocities (SNCV) in control rats ($n = 12$) and rats treated with cisplatin/saline ($n = 12$), cisplatin and 2.0 μg kg^{-1} melanotan-II ($n = 12$) or cisplatin and 20.0 μg kg^{-1} melanotan-II ($n = 12$). The age-matched control rats had significantly higher sensory nerve conduction velocities as compared to the saline-treated cisplatin animals from week 3 onward. Treatment with 20.0 μg kg^{-1} of melanotan-II yielded significant beneficial effects (weeks 3 and 5, $P < 0.05$, ANOVA for repeated measurements with a Bonferroni post-hoc test).

In the age-matched controls, sensory nerve conduction velocity increased to about 64 m s^{-1}, as expected in these not yet fully grown rats. In the cisplatin/saline-treated animals, the sensory nerve conduction velocity did not increase and was significantly lower as compared to the age-matched controls from week 3 onward (a cumulative dose of cisplatin of 12 mg kg^{-1}, ANOVA for repeated measurements followed by a Bonferroni post-hoc test, $P < 0.001$). This indicates that a neuropathy did indeed develop in the cisplatin/saline-treated rats. Treatment with the highest dose of melanotan-II (20 μg kg^{-1}) provided protection against the cisplatin-induced slowing of sensory nerve conduction velocity, as it resulted in significantly faster nerve conduction velocities as compared to that of the cisplatin/saline-treated rats from week 3 onward (ANOVA for repeated measurements followed by a Bonferroni post-hoc test, $P < 0.05$). The lower dose of melanotan-II (2 μg kg^{-1}) did not protect the animals against the cisplatin neuropathy (see Fig. 2).

4. Discussion

In the present study, the efficacy of the potent melanocortin receptor agonist melanotan-II in the treatment of peripheral nerve dysfunction in the rat was investigated. Melanotan-II was found to enhance recovery of sensory function following a crush lesion of the sciatic nerve in the rat. Furthermore, melanotan-II protected against neuropathy induced by cisplatin.

Over the last two decades, many attempts have been made to develop a pharmacotherapy that would enhance recovery of function following brain, spinal cord or peripheral nerve

injury (for review, see Terenghi, 1999; Strand et al., 1994). Among others, we have focused on the possible application of melanocortins in the treatment of peripheral nerve and spinal cord injury (see Gispen et al., 1987). In the present paper, we describe for the first time the neurotrophic and neuroprotective properties of the MSH analog melanotan-II.

As can be seen in Fig. 1, systemic treatment with melanotan-II at a dose of 20 μg kg^{-1} was effective in facilitating sensory recovery following a crush lesion of the sciatic nerve. In comparison, the optimal dose of α-MSH in enhancing recovery of function is 75 μg kg^{-1}. The lower and higher doses of 2 and 50 μg kg^{-1}, respectively, of melanotan-II did not significantly improve the recovery of function. Inverted U-shaped curves have often been observed for in vivo effects of melanocortins (De Koning et al., 1986). At present, this phenomenon is not understood.

It is a well-known fact that the neurotoxicity or ototoxicity remains the major dose-limiting side effect of cisplatin treatment in cancer patients (Alberts and Noel, 1995). Peripheral sensory neuropathy is the primary type of cisplatin neurotoxicity and has been reported in 30–100% of patients, with clinical symptoms typically developing after a cumulative dose of about 300–600 mg m^{-2}, depending on the treatment schedule. The present study for the first time demonstrates that a potent α-MSH analog protects, in a dose-dependent manner, against cisplatin neurotoxicity in an animal model using a high-dose cisplatin treatment schedule; the significantly effective dose of melanotan-II being 20 μg kg^{-1}. These data are comparable to those reported for the beneficial effect of nimodipine (Hamers et al., 1991), glial growth factor-II (Ter Laak et al., 2000b) and the ACTH-(4–9)-analog H-Met(O_2)-Glu-His-Phe-D-Lys-Phe-OH (ORG2766) (De Koning and Gispen, 1987).

Although melanotan-II is one of the most potent melanocortin receptor agonists known to date (Wikberg, 1999), it is still not a particularly selective ligand. Hence, the key question, which melanocortin receptor subtype is involved in vivo peripheral nerve regeneration and protection, can still only be answered by circumstantial evidence. As pointed out in Section 1, in vitro data suggest the involvement of the melanocortin MC_4 receptor subtype (Adan et al., 1999). The currently known distribution of receptor subtype expression in the nervous system also seems to favor this subtype. The melanocortin MC_1 and MC_2 receptors are not expressed in the nervous system, the melanocortin MC_3 receptor is restricted to certain brain regions and the melanocortin MC_5 receptor can be found in several structures in the brain and several non-neuronal structures (for review, see Wikberg, 1999). The melanocortin MC_4 receptor is widely distributed throughout the brain and is the only receptor subtype found in spinal cord and sympathetic ganglia (see Wikberg, 1999; Van der Kraan et al., 1999). Thus, this indirect evidence suggests that the neurotrophic actions of the melanocortins are mediated through the melanocortin MC_4 receptor.

However, when assessing the involvement of the melanocortin MC_4 receptor in nerve regeneration and protection, one would have expected an effectiveness of melanotan-II at a much lower dose as compared to α-MSH, as melanotan-II is a cyclic analog of α-MSH that readily crosses the blood–brain barrier and has a 100× higher affinity for the melanocortin MC_4 subtype than α-MSH. The effective dose of melanotan-II is, however, only three times lower than that of α-MSH.

Therefore, further research is needed to answer the question which specific melanocortin receptor subtype is responsible for the effects of melanocortins on neuronal regeneration and protection in the central and peripheral nervous system.

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