Neuroscience 138 (2006) 293–301

MELANOTAN-II: INVESTIGATION OF THE INDUCER AND FACILITATOR EFFECTS ON PENILE ERECTION IN ANAESTHETIZED RAT

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Abstract—The effects of melanotan-II, a non-specific agonist of melanocortin receptors, on erection and its possible sites of action were investigated in anesthetized rats. Delivered i.v. (0.1, 0.3 and 1 mg/kg) or within the paraventricular nucleus of the hypothalamus (0.1 and 1 μg), melanotan-II exerted a dose-dependent inducer activity on erection by eliciting erectile events and shortening latency of the first erectile event to occur. Erectile events were of higher amplitude in rats treated with melanotan-II i.t. (0.2 μg) delivered at the L6–S1 level than in animals treated with the vehicle i.t. delivered. Erectile responses elicited by cavernous nerve stimulation were increased after i.v. melanotan-II (1 mg/kg), thereby exerting facilitator effect on erection. In contrast, melanotan-II injected within the corpus cavernosum (1 μg) did not display any facilitator activity. To investigate the neural pathways involved in the facilitator effect of melanotan-II, we performed acute spinalization (T8 level) and differential selective nerve transections. Neither spinalization nor bilateral transection of pelvic nerves or dorsal penile nerves impaired facilitator activity of i.v. melanotan-II (1 mg/kg). Conversely, the facilitator effect of melanotan-II was abolished after acute removal of the lumbar paravertebral sympathetic chain. These results lead to the conclusion that central and peripheral melanocortin pathways are recruited by melanotan-II, depending on its route of delivery, to exert both inducer and facilitator activities on erection. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: melanocortin receptor, paraventricular nucleus, spinal cord, sympathetic nervous system.

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Abbreviations: ACTH, adrenocorticotropin hormone; ANOVA, analysis of variance; AUC, area under the curve; EE, erectile event; i.c., intracorporally; ICP, intracavernous pressure; i.t., intrathecal/intrathecally; MAP, mean arterial pressure; α-MSH, alpha-melanocyte stimulating hormone; MT-II, melanotan-II; PVN, paraventricular nucleus; SNK test, Student-Newman-Keul's test; SPN, sacral parasympathetic nucleus.

0306-4522/06$30.00+0.00 © 2005 Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2005.11.008

Penile erection is an integrative and highly coordinated physiological process modulated at different levels of the neuraxis (Andersson, 2000; Giuliano and Rampin, 2004). The penile erectile tissue as well as the arterial supply to the penis receives sympathetic and parasympathetic innervations originating from the spinal cord that exert respectively antierectile and proerectile roles. The balance between the sympathetic and parasympathetic tones determines the functional state of the penis and, when the parasympathetic tone becomes predominant, relaxation of smooth muscle cells of the corpora cavernosa as well as increase of arterial inflow of blood to the erectile tissue occur. This is responsible for a dramatic increase in intracavernosal pressure (ICP). The final result is tumescence followed by rigidity of the penis. In addition, in many mammalian species, intense brief contractions of bulbospongiosus and ischiocavernosus striated muscles located at the penile crus reinforce penile rigidity (Andersson and Wagner, 1995). Local mechanisms of erection are under the control of a variety of neurotransmitters contained in supraspinal and spinal centers and sensory peripheral pathways. Among them, adrenocorticotropin hormone (ACTH) and alpha-melanocyte stimulating hormone (α-MSH) have been reported to enhance penile erection in a range of mammalian species (Argiolas et al., 2000; Hohmann et al., 2000; Vemulapalli et al., 2001).

The melanocortins ACTH and α-MSH are derived from a common 31 kD precursor, the proopiomelanocortin. Melanocortins are produced within the CNS, and act either at central or peripheral sites to regulate a wide variety of physiological functions such as steroidogenesis (Abdel-Malek, 2001), energy homeostasis (Huszar et al., 1997), blood pressure regulation (Dunbar and Lu, 1999), and penile erection (Giuliano, 2004). Five melanocortin receptors, belonging to the G protein-coupled receptor superfamily, have been cloned (Adan and Gispen, 1997). MC_1, MC_2, and MC_5 are not appreciably expressed in the CNS whereas MC_3 and MC_4 are found widely distributed in the rat brain, and more especially in brain nuclei involved in the regulation of penile erection, such as medial preoptic area and paraventricular nucleus (PVN) of the hypothalamus (Roselli-Rehfuss et al., 1993; Mountjoy et al., 1994). Finally, MC_4 mRNA has been detected in rats in dorsal horn of the spinal cord and in dorsal root ganglia (both at the lumbar level) as well as at free nerve endings and mechanoreceptors in both the corpora cavernosa and the glans penis (Mountjoy et al., 1994; Starowicz et al., 2004; Van der Ploeg et al., 2002).

The role of the melanocortinergic system in regulating penile erection has been investigated by using different animal models. ACTH and α-MSH induced penile erection in conscious or anesthetized rats when injected i.c.v. or within the periventricular region in the hypothalamus (Argiolas et al., 2000; Mizusawa et al., 2002). Melanotan-II (MT-II), a potent exogenous nonselective agonist of MC_1, MC_3, MC_4, and MC_5 displayed proerectile activity, i.e. induced penile erection without sexual stimulation, in conscious rats after i.c.v. or intrathecal (i.t.) application (Wessells et al., 2003a,b). However, the fact that the inducer activity of α-MSH on erection was not reversed by i.c.v. coinjection of the preferential MC_4 antagonist (HS014) suggested that this activity was not mediated via MC_4 (Argiolas et al., 2000; Vergoni et al., 1998). On the other hand, Van der Ploeg and colleagues (2002) reported clear evidence supporting the role of MC_4 in modulating erectile function. They showed that a selective MC_4 agonist facilitated the erectile response induced by electrical stimulation of the cavernous nerve in anesthetized mouse, and they further suggested a spinal and/or penile site of action for this compound.

As a whole, the above data indicate that melanocortins exert both facilitator and inducer activities on erection at different levels of the neuraxis that remain to be clearly determined. We thus aimed to clarify this issue by (i) testing the effects of MT-II injected via different routes on erection in anesthetized rat, (ii) using different experimental paradigms to specifically investigate inducer and facilitator activity on erection, and (iii) carrying out differential selective lesions at different levels of the neural circuitry driving pro- or antierectile neural signals.

EXPERIMENTAL PROCEDURES

Animal preparation

All animal experiments were carried out in accordance with the European Communities Council Directives (86/609/EEC) on the use of laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Male Sprague–Dawley rats (Charles-River, L'Arbresle, France), weighing 200–250 g, were used in this study. They were anesthetized with urethane (1.2 g/kg) and placed on a homeothermic blanket to maintain their temperature at 37 °C. A catheter was inserted into the left carotid artery for mean arterial pressure (MAP) monitoring. For ICP monitoring, the penis was denuded of skin and a 25-gauge needle connected to a catheter was inserted into one corpora cavernosa. Arterial and cavernosal catheters were filled with heparinized saline (25 IU/ml) and connected to pressure transducers (EM 750, Elcomatic, Glasgow, UK). The left cavernous nerve was exposed at the lateral aspect of the prostate, with the aid of a dissecting microscope and mounted on a bipolar platinum electrode connected to an electrical stimulator (AMS 2100, Phymep, France). Pressure signals were amplified (Bionic Instruments, Nozay, France), digitized (Labmaster DMA, Scientific Solutions, Solon, OH, USA) and converted to millimeters of mercury (mm Hg) after calibration (Axotape V2, Axon Instruments, Foster City, CA, USA).

Effect of MT-II on erectile activity in anesthetized rats

To investigate the inducer activity on erection, MT-II or vehicle (saline) was acutely injected i.v., i.t. or within the PVN after a 5 min baseline recording period was obtained. ICP and MAP were then recorded for a 60 min period after saline or MT-II delivery. I.v. injections (three doses; 0.1, 0.3, and 1 mg/kg in saline) were performed with a catheter inserted in the jugular vein. For i.t. injections, a catheter was inserted through the atlanto-occipital membrane and directed caudally. Ten minutes after i.t. catheter insertion, penile and pelvic surgery began. One dose (0.2 μg in 10 μl saline) was tested. Correct implantation of the caudal tip of the catheter at the T12–L1 or L4–L6 spinal cord level was checked by complete laminectomy after kill of the animal. Rostrocaudal diffusion of i.t. injection was checked in two rats delivered with Evan's Blue (10 μl) at the L6–S1 level. For PVN injection experiments, penile and cavernous nerve dissections were performed and then the rat was placed on a stereotaxic frame. Injection (two doses; 0.1 and 1 μg in 1 μl saline) was performed using a Hamilton microsyringe 20 min after its positioning within the PVN (coordinates: 0.3 mm posterior to bregma, 0.4 mm lateral to the midline, 8.4 mm below the skull surface, with the upside of the incisor bar at the level of the interaural line). After kill, the brain was removed and serially cut in 20 μm thick sections on a cryostat. One in five slices was mounted on gelatinized slides and dyed with Cresyl Violet in order to check the position of the injection tip. Rats found to have the tip of the microsyringe needle outside the PVN were rejected from the study. For each experiment, control of ICP recording conditions was performed by stimulating the cavernous nerve (30 s overall duration, 6 V, 10 Hz, 1 ms pulse) at the end of each experimental period to elicit a complete erectile response.

Effect of MT-II on erection elicited by neurostimulation in anesthetized rats

To investigate the facilitator activity of MT-II on erection, erectile responses were elicited by electrical stimulation of the cavernous nerve at various submaximal frequencies eliciting a partial erectile response (45 s overall duration, 6 V, 1 ms pulse and either 0.5, 1, 3 and 5 Hz). Each electrical stimulation was performed twice, in a random order, every 90 s, before and after the i.v. injection of MT-II (0.3 and 1 mg/kg) or saline. This protocol was used in control rats and in rats immediately after spinalization at the T8 level or peripheral nerve transection. For spinalization, the T8 spinal cord was exposed through a laminectomy of the T7–T8 vertebrae and, after dura incision, a complete transversal section of the underlying T8 spinal cord was performed. All peripheral nerve transections were performed bilaterally. Pelvic nerves, which convey the proerectile parasympathetic fibers issued from the sacral parasympathetic nucleus (SPN) to the penis, were freed from the surrounding connective tissue on the lateral aspect of the prostate and sectioned 5 mm posterior to the major pelvic ganglion. Dorsal nerves of the penis, that convey sensory fibers from the penis to the spinal cord, were freed as proximally as possible at the base of the penis and transected. Both trunks of the lumbar paravertebral sympathetic chain, harboring the sympathetic efferent fibers innervating the pelvis, were removed at the L4–L5 level together with the inferior mesenteric ganglia. The different sites of acute neural lesions are summarized on Fig. 1.

A different protocol was used to investigate the facilitator activity of MT-II when delivered within the corpora cavernosa, i.e. intracorporally (i.c.). Erectile responses were elicited by electrical stimulation of the cavernous nerve at two different submaximal frequencies (45 s overall duration, 6 V, 1 ms pulse and either 3 Hz and 5 Hz) in order to elicit partial penile tumescence. For each frequency, two electrical stimulations were performed before and 30 s after the i.c. delivery of 1 μg MT-II in 100 μl saline or the corresponding volume of saline. I.c. injections were performed via the needle used for ICP measurement.

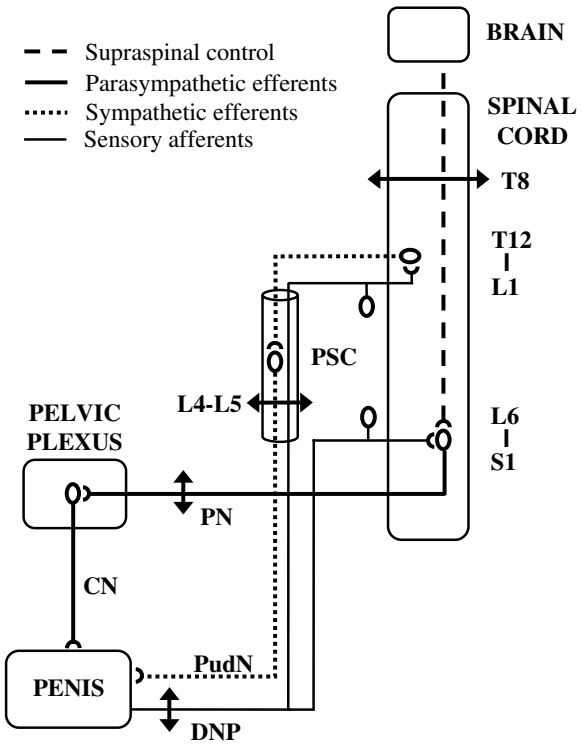

Fig. 1. Diagrammatic illustration of the differential selective neural lesions acutely performed in anesthetized rats. Sites of sectioning are represented by double head arrows. Abbreviations: CN, cavernous nerve; DNP, dorsal nerve of the penis; PudN, pudendal nerve; PN, pelvic nerve; PSC, paravertebral sympathetic chain.

Drugs

MT-II (purity, 98.4%) was purchased from Bachem (Voisins-le-Bretonneux, France). All other reagents were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France).

Data and statistical analysis

Effect of MT-II on erectile activity in anesthetized rats. Only increases in ICP superior to i) the sum of the average+3 standard deviations of the basal ICP (mean ICP measured during the first 5 min of recording before treatment) and ii) an absolute value of 20 mm Hg were quantified. In anesthetized rats, such ICP rises correspond to erectile events (EEs) (Chen et al., 1992; Giuliano et al., 1993). Their number was averaged for each experimental condition. Amplitude of ICP increase during an EE was divided by the corresponding MAP to normalize their dependence on MAP (Giuliano et al., 1993). ICPmax/MAP, expressed as percentage, corresponded to the maximal value reached by ICP during the EE divided by the MAP calculated for the duration of the EE. The ratio of area under the curve (AUC) of the ICP and the corresponding MAP (AUC/MAP, mm Hg·s), which reflects both amplitude and duration of the EE, was also determined. ICPmax/MAP and AUC/MAP were averaged per rat, and then per group. Rats which did not display any EE (non-responding rats) during the recording period were attributed the value 0 for the number of EEs, and ICPmax/MAP and AUC/MAP were considered as missing data for the statistical analysis. The lag time separating the end of the drug injection from the occurrence of the first EE (latency) was determined for each rat and averaged for each experimental condition.

Effect of MT-II on erection elicited by neurostimulation in anesthetized rats. Increase in ICP elicited by electrical stimulation of the cavernous nerve was computed from the basal ICP, and normalized by the corresponding MAP for the duration of the electrical stimulation (Δ ICPmax/MAP). The ratio AUC/MAP was also calculated for each ICP increase induced by stimulation of the cavernous nerve. Δ ICPmax/MAP and AUC/MAP were averaged for each frequency of electrical stimulation of the cavernous nerve before and after MT-II or saline delivery for each rat. The post-treatment values of Δ ICPmax/MAP and AUC/MAP were then expressed as the % of the pre-treatment values (%Δ ICPmax/MAP and %AUC/MAP) for each rat and for each frequency of electrical stimulation. %Δ ICPmax/MAP and %AUC/MAP for each frequency was then averaged for each experimental condition.

Statistical analysis

Results obtained in the different experimental conditions were compared with *t*-test or one-way ANOVA analysis of variance (ANOVA). One-way ANOVA was followed by Student-Newman-Keul's test (SNK test) when applicable. Differences were considered significant when $P<0.05$. Results were expressed as mean±standard error of the mean.

RESULTS

Effect of MT-II on erectile activity in anesthetized rats

In anesthetized rats, EEs were characterized by transient increase of ICP (Fig. 2). EEs were observed in two of nine rats in the saline group, and in three of nine, four of eight and seven of nine rats in the groups treated with 0.1, 0.3 and 1 mg/kg MT-II i.v. respectively. No significant changes in the mean number of EEs were obtained after MT-II i.v. injections (Table 1). The amplitude of EEs occurring during the one-hour period of recording was similar after saline or MT-II at the three dosings tested (Fig. 3a) whereas the AUC/MAP ratio was significantly increased for MT-II at 1 mg/kg ($F[3,36]=3.67$, $P<0.05$; SNK test, $P<0.05$; Fig. 3b). MT-II delivery resulted in a significant decrease of the latency for the first EE to occur at the three dosings tested ($F[3,32]=7.27$, $P<0.001$; SNK test, $P<0.05$) compared with the saline group (Table 1). A posteriori SNK test yielded no significant difference between the three dosings tested.

After intra-PVN delivery of MT-II or saline, EEs were observed in four of eight rats in the saline group, and in four of eight and eight of eight rats in the groups treated with 0.1 and 1 μg respectively. The mean number of EEs was significantly increased upon injection of MT-II in the PVN ($F[2,21]=10.8$, $P<0.001$; Table 1). This increase was dose-dependent, and significant for the MT-II 1 μg treated group compared with the MT-II 0.1 μg and saline groups ($P<0.01$ and $P<0.001$ respectively, SNK test). Amplitude of EEs occurring after delivery of MT-II in the PVN was not statistically increased compared with saline (Fig. 3a). During EEs, the increase in ICP lasted longer in the group treated with 1 μg MT-II as reflected by the significant augmentation of AUC/MAP ratio (+79% compared with saline, $F[2,21]=5.4$, $P<0.05$; SNK test, $P<0.05$; Fig. 3b). In the two groups receiving intra-PVN injection of MT-II, latency was significantly decreased compared with the corresponding saline injection ($F[2,21]=23.8$, $P<0.001$; SNK test, $P<0.001$; Table 1).

To further confirm the supraspinal inducer activity of MT-II, a group of seven rats was injected i.v. with 1 mg/kg MT-II after acute spinalization at the T8 level. The effect of

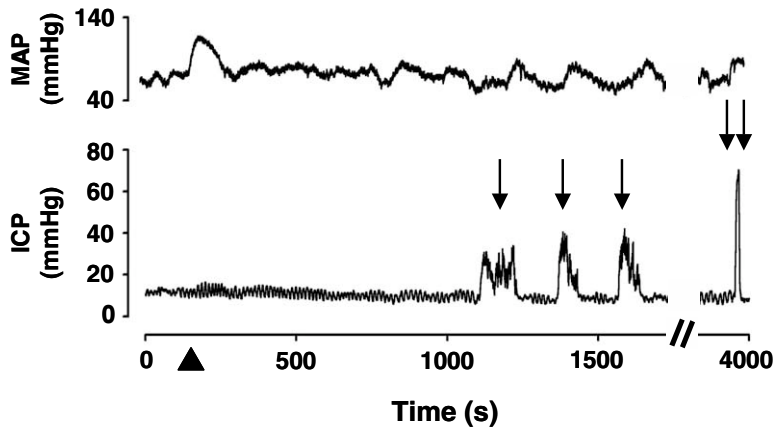

Fig. 2. Recordings of intracavernous and blood pressure (ICP and MAP respectively) upon i.v. MT-II delivery. ICP (lower trace) and MAP (upper trace) were recorded upon i.v. injection of 0.3 mg/kg MT-II (arrowhead in abscissa) in urethane-anesthetized rats. EEs appeared as transient increase of ICP (arrows). A complete erectile response was induced at the end of the experiment (double arrow) by electrical stimulation of the cavernous nerve as positive control.

MT-II was almost completely suppressed; only two of seven rats exhibited at least one EE during the 60 min period following injection.

Upon control i.t. injection with Evan's Blue at L6–S1 level, the most intense coloration was observed at the distal tip of the catheter. Caudal spreading of the dye appeared limited with a weak coloration detected at the S2 level. Rostral diffusion occurred along the external part of the catheter with a weak coloration detected up to the T10 level. MT-II (0.2 μg) or saline was delivered i.t. at the L6–S1 spinal level in two groups of six rats (five of six and four of six rats respectively exhibiting at least one EE). The number of EEs was not significantly greater in the group injected with MT-II compared with saline (Table 1; *t*-test, $P=0.06$). Amplitude of EEs as well as AUC/MAP ratio was significantly increased when MT-II was i.t. delivered at the L6–S1 spinal level compared with a corresponding saline injection (ICPmax/MAP, *t*-test, $P<0.05$; Fig. 3a; AUC/MAP, *t*-test, $P<0.05$; Fig. 3b). The latency was not significantly decreased in the MT-II group (Table 1).

To investigate whether pelvic nerves constitute the efferent neural pathway recruited by MT-II when eliciting EE upon i.t. delivery (0.2 μg) at the L6–S1 level, pelvic nerves were bilaterally transected in four rats. Acute transection of the pelvic nerves completely abolished the occurrence of EEs in these four animals.

MT-II (0.2 μg) was also delivered at the T12–L1 spinal level in seven rats, where it did not elicit a significant proerectile activity (Table 1; ICPmax/MAP: 47±4%; AUC/MAP: 10.6±1.3 s).

Table 1. Effects of MT-II injected via different routes on the number of EEs and latency of the first EE

Route of injection	Treatment	EEs number	Latency (s)
Intravenous (Eight to nine animals)	Saline	0.8±0.4	2381±304
	MT-II 0.1 mg/kg	1.1±0.6	1088±573*
	MT-II 0.3 mg/kg	2.7±1.2	632±336*
	MT-II 1 mg/kg	2.2±0.8	1151±445*
Intra-PVN (Eight to nine animals)	Saline	1.0±0.5	1203±187
	MT-II 0.1 μg	2.8±1.2	410±141*
	MT-II 1 μg	7.7±1.1*	316±59*
Intrathecal (L6–S1) (Six animals)	Saline	1.9±1.1	1217±507
	MT-II 0.2 μg	5.8±1.2	701±351
Intrathecal (T12–L1) (Seven animals)	Saline	1.5±1.0	1429±618
	MT-II 0.2 μg	4.0±1.2	909±314

Statistics: one-way ANOVA and SNK test.
* $P<0.05$ compared to saline.

Effect of MT-II on erection elicited by neurostimulation in anesthetized rats

Electrical stimulation of the cavernous nerve at 0.5 or 1 Hz, 6 V, 1 ms pulse for 45 s elicited increases in ICP of low amplitude, leading to a poor signal to noise ratio and an unreliable quantification of erectile response parameters. Increasing the frequency of electrical stimulations led to obtaining more robust erectile responses, and eventually 3 and 5 Hz were considered for quantitative investigation of the facilitator activity of MT-II.

Electrical stimulations of the cavernous nerve were performed in three groups of 10 rats treated i.v. with saline, 0.3 or 1 mg/kg MT-II. At 5 Hz, %Δ ICPmax/MAP was significantly increased by 1 mg/kg MT-II compared with a corresponding injection of saline (127±12% and 89±5% respectively, *t*-test, $P<0.01$; Fig. 4a). Similarly %AUC/MAP was found higher after i.v. delivery of 1 mg/kg MT-II (130±9% versus 88±4% for saline, *t*-test, $P<0.05$; Fig. 4b). At 3 Hz, injection of 1 mg/kg MT-II also induced a significant increase of %Δ ICPmax/MAP and %AUC/MAP compared with saline (%Δ ICPmax/MAP, 129±17% and 91±9% respectively, *t*-test, $P<0.05$; Fig. 4a; %AUC/MAP, 123±10% and 91±8% respectively, *t*-test, $P<0.05$; Fig. 4b). Whether cavernous nerve electrical stimulation was applied at 3 or 5 Hz, *t*-test did not yield significant differences between 0.3 and 1 mg/kg MT-II.

Experiments were then performed to determine the site of action for the facilitator effect of MT-II on erection. We first assessed whether the site of action of MT-II to facilitate erection resides within the penis itself in two groups of six rats.

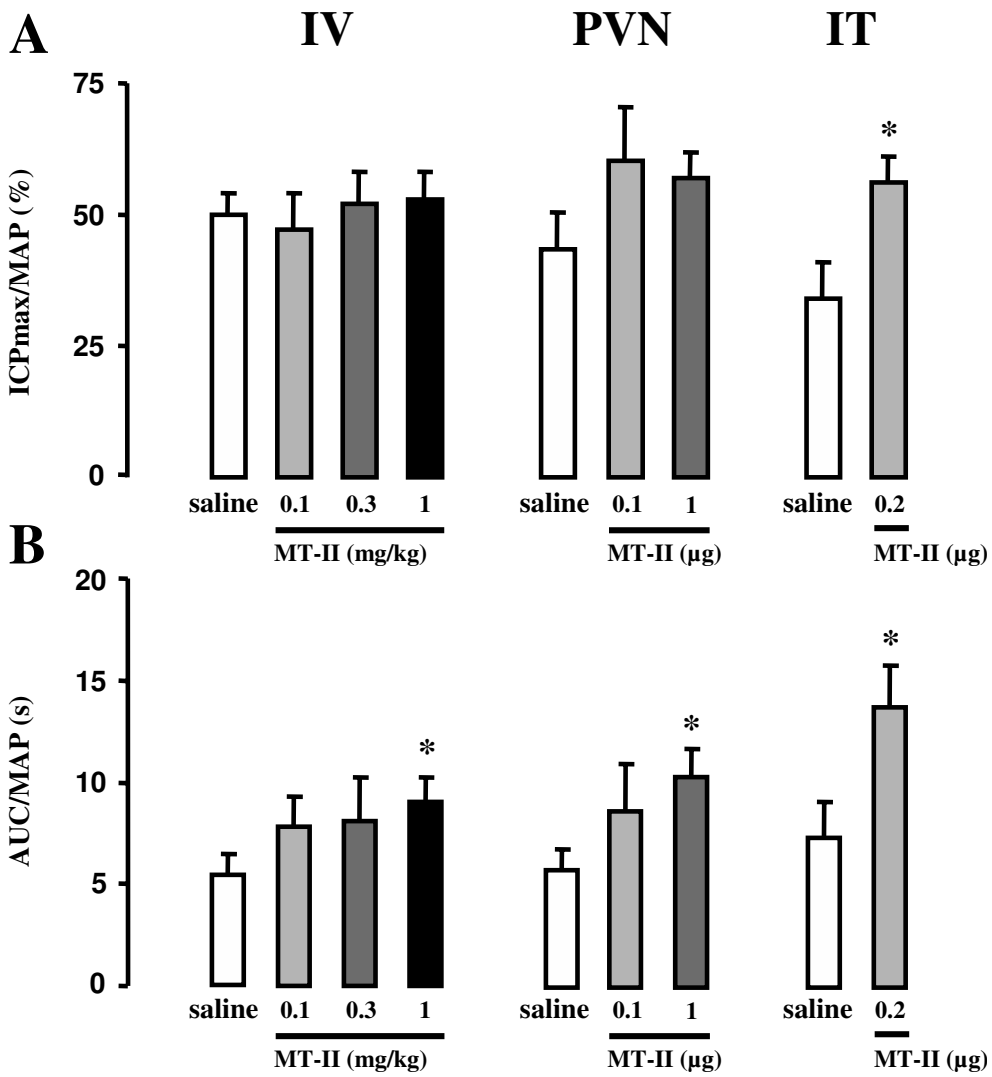

Fig. 3. Quantification and comparison of inducer activity on erection of i.v., i.t. (L6–S1), and intra-PVN delivery of MT-II. Groups of urethane-anesthetized rats were injected either i.v. (left column), in the PVN (middle column) or i.t. (right column) with saline or MT-II (dosings in abscissa). The maximal intracavernosal pressure (ICPmax) (a) reached during EE and the AUC (b) were normalized by the MAP measured during the corresponding EE. Statistics: one-way ANOVA among the four groups injected i.v. (eight to nine rats per group) and the three groups injected in the PVN (eight to nine rats per group), and *t*-test for the two groups injected i.t. (six rats per group). * $P<0.05$ compared with the saline group.

Intracavernosal injection (1 µg in 100 µl) of MT-II failed to increase the erectile response to submaximal electrical stimulation of the cavernous nerve at 3 and 5 Hz (Fig. 4a,b).

We therefore carried out acute spinal cord transection at the T8 level and differential selective nerve transections. A significant facilitator effect of MT-II i.v. (1 mg/kg) was still present after T8 spinalization as shown by a significant increase in %Δ ICPmax/MAP at 3 Hz (173±23% after MT-II ($n=9$) versus 115±15% after saline injection ($n=9$), *t*-test, $P<0.05$) and 5 Hz (%Δ ICPmax/MAP, 150±11% after MT-II versus 107±7% after saline injection, *t*-test, $P<0.05$; Fig. 5). Concerning %AUC/MAP, statistical tests yielded significance only when 5 Hz electrical stimulation was applied to the cavernous nerve (190±29% after MT-II versus 98±9% after saline injection, *t*-test, $P<0.05$).

Acute bilateral transection of the pelvic nerves did not impair the facilitator effect of 1 mg/kg MT-II i.v. delivered as demonstrated by a significant raise of %Δ ICPmax/MAP (at 3 Hz, 195±18% after MT-II ($n=8$) versus 141±11% after saline injection ($n=9$), *t*-test, $P<0.05$; at 5 Hz, 188±17% after MT-II versus 127±13% after saline injection, *t*-test, $P<0.01$) and %AUC/MAP (at 3 Hz, 182±14% after MT-II versus 133±9% after saline injection, *t*-test, $P<0.01$; at 5 Hz, 174±9% after MT-II versus 131±13% after saline injection, *t*-test, $P<0.05$; Fig. 5). In the same way, acute bilateral transection of the dorsal nerves of the penis did not impair the facilitator activity of 1 mg/kg i.v. MT-II as revealed by a significant increase in %Δ ICPmax/MAP (at 3 Hz, 145±12% after MT-II ($n=9$) versus 105±7% after saline injection ($n=9$), *t*-test, $P<0.01$; at 5 Hz, 127±10% after MT-II versus 94±2% after saline injection, *t*-test, $P<0.01$) and %AUC/MAP (at

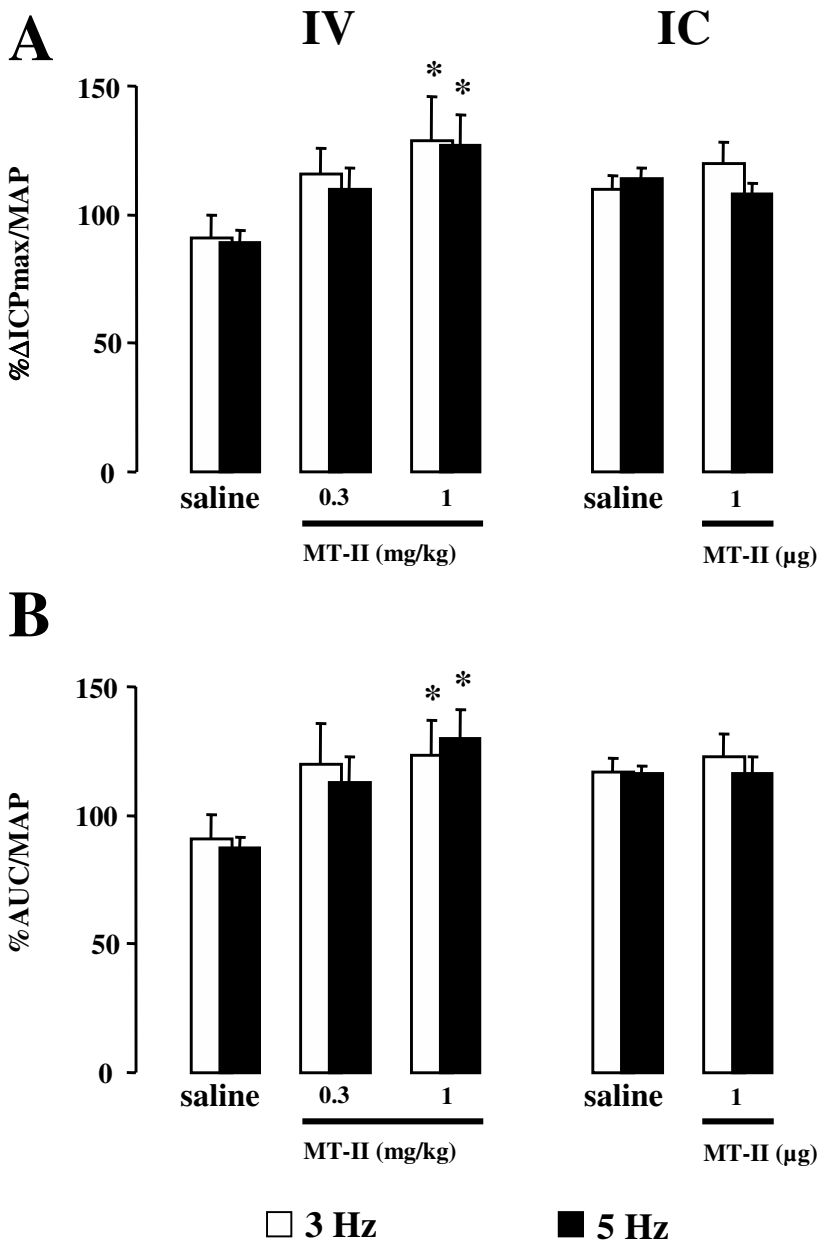

Fig. 4. Effects of MT-II injected i.v. or intracavernosally (i.c.) on the normalized maximal intracavernosal pressure (%ΔICPmax/MAP) (a) and AUC (%AUC/MAP) raises (b) induced by electrical stimulation at 3 and 5 Hz of the cavernous nerve. Statistics: one-way ANOVA (10 rats per group), * $P<0.05$ compared with the saline group.

3 Hz, 138±8% after MT-II versus 101±5% after saline injection, *t*-test, $P<0.01$; at 5 Hz, 130±8% after MT-II versus 96±2% after saline injection, *t*-test, $P<0.01$; Fig. 5).

In contrast, bilateral removal of the paravertebral sympathetic chain between the L4 and L6 levels abolished the facilitator effect of 1 mg/kg MT-II i.v. delivery in 10 rats; %Δ ICPmax/MAP and %AUC/MAP changes were not anymore significant when compared with saline ($n=10$ rats) (Fig. 5).

DISCUSSION

MT-II, a non-specific melanocortin agonist targeting MC_1, MC_3, MC_4 and MC_5 (Al-Obeidi et al., 1989), has been shown to induce erection upon i.v., i.t., and i.c.v. delivery in conscious rats (Wessells et al., 2003a,b). The present report further demonstrates that MT-II also exerts inducer activity in urethane-anesthetized rats following i.v. and intra-PVN delivery. This extends the notion obtained with melanocortins, oxytocin and D2-like dopaminergic agonists that rats under urethane anesthesia represent a valid experimental model for evidencing proerectile activity of drugs (Chen et al., 1999; Giuliano et al., 2001).

A cerebral site of action for MT-II to exert an inducer effect on erection was demonstrated by its intra-PVN delivery (1 μg) that elicited erection in anesthetized rats. This

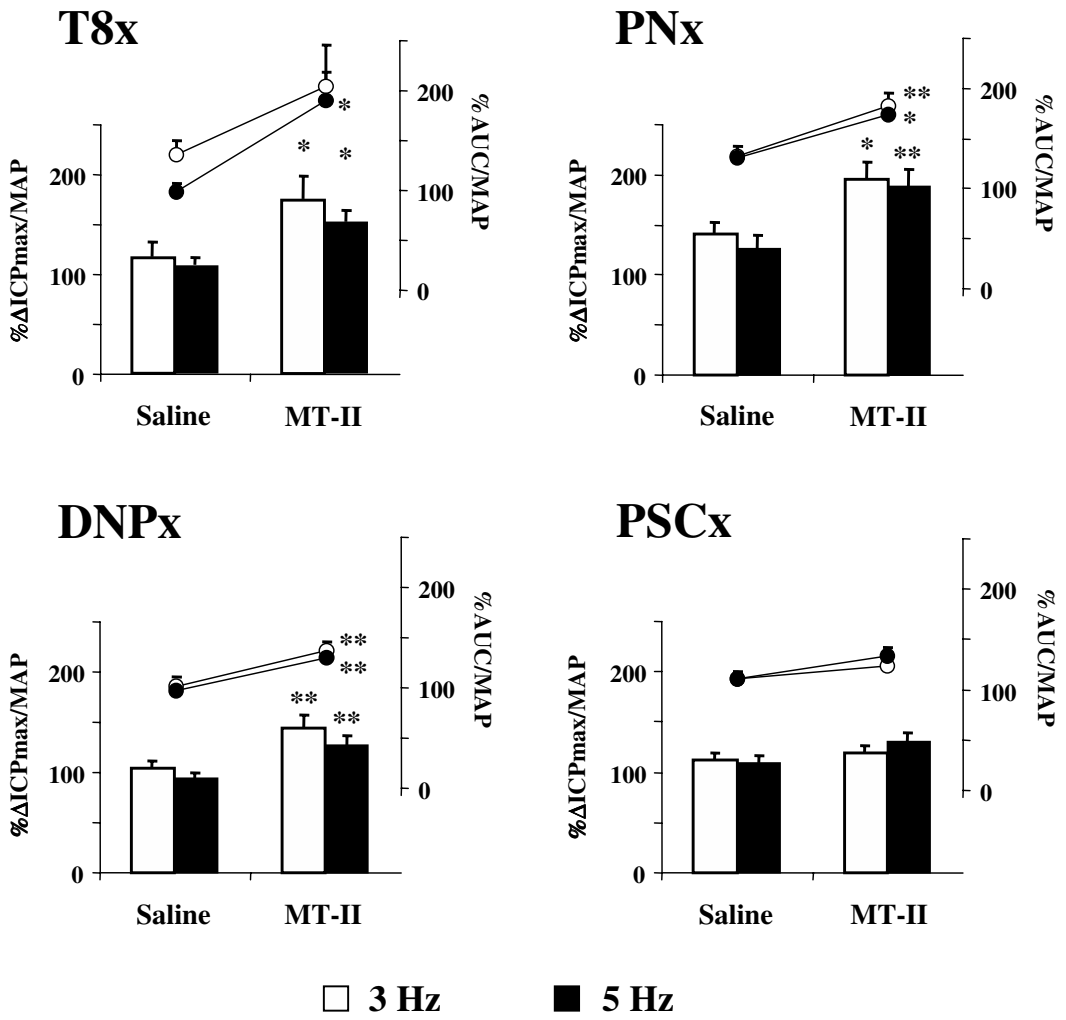

Fig. 5. Determination of the site of action for the facilitator activity of MT-II on erection. The facilitator activity of MT-II was assessed in different conditions. Electrical stimulations (3 and 5 Hz) of the cavernous nerve were performed in rats with spinal cord section at the T8 level (T8x), after bilateral transection of the pelvic nerves (PNx) or dorsal nerves of the penis (DNPx), or after lumbosacral paravertebral sympathetic chain removal (PSCx), before and after the i.v. injection of 1 mg/kg MT-II or the corresponding volume of saline. The results of ΔICPmax/MAP (histograms) and AUC/MAP (dots) increases are the mean of eight to 10 rats. Statistics: *t*-test, * $P<0.05$, ** $P<0.01$ compared with the saline group.

was further supported by abolition of the inducer effect when MT-II was i.v. injected in T8 spinalized rats. This is in agreement with previous experiments (Argiolas et al., 1987, 2000) that furthermore suggested the brain site of action for melanocortin agonists to induce erection as comprising the entire periventricular region. Published data obtained in conscious rats using the preferential MC_4 antagonist HS014 i.c.v. injected suggested that MC_4 does not mediate penile erections induced by melanocortin agonists delivered i.c.v. or in the hypothalamus (Argiolas et al., 2000; Vergoni et al., 1998). Therefore, it may be postulated that stimulation of cerebral MC_3 is more likely responsible for the inducer activity of melanocortinergic agonists.

In the present study in anesthetized rats, MT-II also elicited erection when delivered at both the thoracolumbar (T12–L1) and lumbosacral (L6–S1) levels of the spinal cord, which correspond to the levels of, respectively, the sympathetic and parasympathetic nuclei involved in the spinal command of penile erection (Rampin et al., 1997). This confirms the previous findings obtained in conscious rats (Wessells et al., 2003a,b). Using Evan's Blue for checking diffusion of i.t. injection, an appreciable coloration was detected surrounding the catheter up to T10 level. However, because the inducer effect of MT-II delivered at the L6–S1 level was abolished after section of the pelvic nerves mainly conveying parasympathetic fibers originating from the SPN located at the L6–S1 level in rats we assume that the amount of MT-II reaching more rostral spinal segments (especially T12–L1) following L6–S1 delivery was not responsible for inducing erection. Moreover, as the caudal spreading of i.t. injection appeared limited, we also assume that i.t. delivery of MT-II at the T12–L1 level did not affect the SPN. The MC_4 is the only MC receptor that has been located in the spinal cord (Van der Kraan et al., 1999). This implies that whereas MC_3 is postulated to be responsible for the inducer activity of MT-II delivered intra-PVN, MC_4 is responsible for the inducer activity of i.t. application of MT-II.

We also found that MT-II exerted a facilitator activity on erection, in an experimental paradigm that is typically used to assess the efficacy of peripherally acting drugs enhancing penile erection; the prototype being phosphodiesterase type 5 inhibitors (Giuliano et al., 2003). Using the same experimental paradigm than in the present study, the facilitator effect of tetrahydroisoquinoline, a selective MC_4 agonist, has been demonstrated in anesthetized mice (Van der Ploeg et al., 2002). However, tetrahydroisoquinoline has a poor brain penetration (Martin et al., 2002) and its precise site of action remains unclear. As shown by our results reporting the lack of a facilitator effect of MT-II upon injection into the corpus cavernosum (1 μg), an intracorporal site of action for MT-II is likely ruled out. This is in agreement with Wessells et al. (2003a,b) findings even reporting an inhibition of the increase in ICP elicited by stimulation of the cavernous nerve when i.c. injecting MT-II (100 μg). Despite the report of expression of MC_4 within the glans penis (Van der Ploeg et al., 2002), involvement of sensory afferents originating within the penis in the facilitator activity of MT-II seems also unlikely as shown in the present study by the lack of effect of acute bilateral dorsal penile nerves transection. Nevertheless, to definitely rule out any participation of afferent components on facilitator activity of MT-II, its effect on reflexogenic erection using a suitable experimental model remains to be investigated. On the other hand, it is noteworthy that transection of the pelvic nerves did not significantly alter the facilitator activity of MT-II when delivered i.v., making the involvement of the parasympathetic pathway in the facilitator activity of MT-II unlikely. The fact that the same lesion was effective in suppressing the inducer activity of MT-II i.t. application (L6–S1 level) can be emphasized here.

In contrast, the abolition of the facilitator activity of i.v. injected MT-II after removal of the lumbosacral paravertebral sympathetic suggests that melanocortinergic system modulates the tone of the sympathetic efferents to the pelvis. This phenomenon has already been reported following i.c.v. delivery of MT-II (Rahmouni et al., 2003; Haynes et al., 1999). It is more problematic to understand how an increase of sympathetic activity leads to facilitate penile erection. When penile erection is induced by electrical stimulation of the medial preoptic area in anesthetized rats, sympathetic fibers running in the paravertebral sympathetic chain are recruited and have been postulated to be responsible for vasoconstriction of pelvic arteries not supplying the erectile tissue thereby resulting in diverting blood toward the penis and facilitating erection by this means (Giuliano et al., 1997). The activation by MT-II of a subgroup of sympathetic neurones, which axons are conveyed by the lumbar paravertebral sympathetic chain, may very well play therefore a proerectile role. Further investigations are required to confirm this hypothesis.

CONCLUSION

In conclusion, the present work shows that central and peripheral melanocortin pathways, depending on the route of delivery, might be recruited by MT-II to exert both inducer and facilitator activities on penile erection. The multiple sites of action for MT-II to exert its proerectile effect support further research for the use of melanocortin agonists for the treatment of erectile dysfunction.

REFERENCES

Abdel-Malek ZA (2001) Melanocortin receptors: their functions and regulation by physiological agonists and antagonists. Cell Mol Life Sci 58:434–441.

Adan RA, Gispen WH (1997) Brain melanocortin receptors: from cloning to function. Peptides 18:1279–1287.

Al-Obeidi F, de la Castrucci AM, Hadley ME, Hruby VJ (1989) Potent and prolonged acting cyclic lactam analogues of α-melanotropin: design based on molecular dynamics. J Med Chem 32:2555–2561.

Andersson KE (2000) Neurotransmitters: central and peripheral mechanisms. Int J Impot Res 12:S26–S33.

Andersson KE, Wagner G (1995) Physiology of erection. Physiol Rev 75:191–236.

Argiolas A, Melis MR, Mauri A, Gessa GL (1987) Paraventricular nucleus lesion prevents yawning and penile erection induced by apomorphine and oxytocin but not by ACTH in rats. Brain Res 421:349–352.

Argiolas A, Melis MR, Murgia S, Schiot HB (2000) ACTH- and alpha-MSH-induced grooming, stretching, yawning and penile erection in male rats: site of action in the brain and role of melanocortin receptors. Brain Res Bull 51:425–431.

Chen KK, Chan JY, Chang LS, Chen MT, Chan SH (1992) Intracavernous pressure as an experimental index in a rat model for the evaluation of penile erection. J Urol 147:1124–1128.

Chen KK, Chan JY, Chang LS (1999) Dopaminergic neurotransmission at the paraventricular nucleus of hypothalamus in central regulation of penile erection in the rat. J Urol 162:237–242.

Dunbar JC, Lu H (1999) Leptin-induced increase in sympathetic nervous and cardiovascular tone is mediated by proopiomelanocortin (POMC) products. Brain Res Bull 50:215–221.

Giuliano F (2004) Control of penile erection by the melanocortinergic system: experimental evidences and therapeutic perspectives. J Androl 25:683–691.

Giuliano F, Bernabe J, Alexandre L, Niewoehner U, Haning H, Bischoff E (2003) Pro-erectile effect of vardenafil: in vitro experiments in rabbits and in vivo comparison with sildenafil in rats. Eur Urol 44:731–736.

Giuliano F, Bernabe J, Brown K, Droupy S, Benoit G, Rampin O (1997) Erectile response to hypothalamic stimulation in rats: role of peripheral nerves. Am J Physiol 273:R1990–R1997.

Giuliano F, Bernabe J, Jardin A, Rousseau JP (1993) Antierectile role of the sympathetic nervous system in rats. J Urol 150:519–524.

Giuliano F, Bernabe J, McKenna K, Longueville F, Rampin O (2001) Spinal proerectile effect of oxytocin in anesthetized rats. Am J Physiol 280:R1870–R1877.

Giuliano F, Rampin O (2004) Neural control of erection. Physiol Behav 83:189–201.

Haynes WG, Morgan DA, Djalali A, Sivitz WI, Mark AL (1999) Interactions between the melanocortin system and leptin in control of sympathetic nerve traffic. Hypertension 33:542–547.

Hohmann JG, Teal TH, Clifton DK, Davis J, Hruby VJ, Han G, Steiner RA (2000) Differential role of melanocortins in mediating leptin's central effects on feeding and reproduction. Am J Physiol 278: R50–R59.

Huszar D, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q, Berkemeier LR, Gu RA, Kesterson RA, Boston BA, Cone RD, Smith FJ, Campfield LA, Burn P, Lee F (1997) Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell 88:131–141.

Martin WJ, McGowan E, Cashen DE, Gantert LT, Drisko JE, Hom G, Nargund R, Sebhat I, Howard AD, Van der Ploeg L, Macintyre DE

(2002) Activation of melanocortin MC4 receptors increases erectile activity in rats ex copula. Eur J Pharmacol 454:71–79.

Mizusawa H, Hedlund P, Andersson KE (2002) Alpha-melanocyte stimulating hormone and oxytocin induced penile erections and intracavernous pressure increases in the rat. J Urol 167:757–760.

Mountjoy KG, Mortrud MT, Low MJ, Simerly RB, Cone RD (1994) Localization of the melanocortin-4 receptor (MC4-R) in neuroendocrine and autonomic control circuits in the brain. Mol Endocrinol 8:1298–1308.

Rahmouni K, Haynes WG, Morgan DA, Mark AL (2003) Role of melanocortin-4 receptors in mediating renal sympathoactivation to leptin and insulin. J Neurosci 23:5998–6004.

Rampin O, Bernabe J, Giuliano F (1997) Spinal control of penile erection. World J Urol 15:2–13.

Roselli-Rehfuss L, Mountjoy KG, Robbins LS, Mortrud MT, Low MJ, Tatro JB, Entwistle ML, Simerly RB, Cone RD (1993) Identification of a receptor for gamma melanotropin and other proopiomelanocortin peptides in the hypothalamus and limbic system. Proc Natl Acad Sci USA 90:8856–8860.

Starowicz K, Bilecki W, Sieja A, Przewlocka B, Przewlocki R (2004) Melanocortin 4 receptor is expressed in the dorsal root ganglions and down-regulated in neuropathic rats. Neurosci Lett 358:79–82.

Van der Kraan M, Tatro JB, Entwistle ML, Brakkee JH, Burabch JP, Adan RA, Gipsen WH (1999) Expression of melanocortin receptors and pro-opiomelanocortin in the rat spinal cord in relation to neurotrophic effects of melanocortins. Mol Brain Res 63:276–286.

Van der Ploeg LH, Martin WJ, Howard AD, Nargund RP, Austin CP, Guan X, Drisko J, Cashen D, Sebhat I, Patchett AA, Figueroa DJ, Dilella AG, Connolly BM, Weinberg DH, Tan CP, Palyha OC, Pong SS, Macneil T, Rosenblum C, Vongs A, Tang R, Yu H, Sailer W, Fong TM, Huang C, Tota MR, Chang RS, Stearns R, Tamvakopoulos C, Christ G, Drazen DL, Spar BD, Nelson RJ, Macintyre DE (2002) A role for the melanocortin 4 receptor in sexual function. Proc Natl Acad Sci USA 99:11381–11386.

Vemulapalli R, Kurowski S, Salisbury B, Parker E, Davis H (2001) Activation of central melanocortin receptors by MT-II increases cavernosal pressure in rabbits by the neuronal release of NO. Br J Pharmacol 134:1705–1710.

Vergoni AV, Bertolini A, Mutulis F, Wikberg JE, Schioth HB (1998) Differential influence of a selective melanocortin MC4 receptor antagonist (HS014) on melanocortin-induced behavioural effects in rats. Eur J Pharmacol 362:95–101.

Wessells H, Hruby VJ, Hackett J, Han G, Balse-Srinivasan P, Vanderah TW (2003a) MT-II induces penile erection via brain and spinal mechanisms. Ann N Y Acad Sci 994:90–95.

Wessells H, Hruby VJ, Hackett J, Han G, Balse-Srinivasan P, Vanderah TW (2003b) Ac-Nle-c[Asp-His-Dphe-Arg-Trp-Lys]-NH2 induces penile erection via brain and spinal melanocortin receptors. Neuroscience 118:755–762.

(Accepted 7 November 2005)
(Available online 19 December 2005)