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Activation of melanocortin MC_4 receptors increases erectile activity in rats *ex copula*

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Abstract

Melanocortin peptide agonists, α-melanocyte stimulating hormone (α-MSH) and melanotan-II, stimulate erectile activity in a variety of species, including man. Since neither peptide discriminates amongst melanocortin receptors, it is not clear which subtype mediates these pro-erectile effects. Here, we present data that melanocortin-induced erectogenesis is mediated by melanocortin MC_4 receptors. Systemic administration of a melanocortin MC_4 receptor agonist (*N*-[(3*R*)-1,2,3,4-tetrahydroisoquinolinium-3-ylcarbonyl]-(1*R*)-1-(4-chlorobenzyl)-2-[4-cyclohexyl-4-(1*H*-1,2,4-triazol-1ylmethyl)piperidin-1-yl]-2-oxoethylamine; THIQ) with high selectivity over other melanocortin receptors enhanced intracavernosal pressure and stimulated erectile activity in rats *ex copula*. THIQ dose-dependently (1–5 mg/kg, i.v.) increased the total number of erections, to an extent comparable or greater than that produced by apomorphine (0.025 mg/kg, s.c.). Central administration of THIQ (20 μg, intracerebroventricular (i.c.v.)) increased the number of reflexive penile erections; whereas administration of both a nonselective endogenous melanocortin MC_4 receptor antagonist (agouti-related protein (AgRP), 5.5. μg, i.c.v.) and a melanocortin MC_4 receptor preferring antagonist (MPB10, 1 mg/kg, i.v.) blocked THIQ-induced erectogenesis. These pro-erectile effects were also attenuated by systemic or central administration of an oxytocin antagonist (L-368899, 1 mg/kg, i.v.). Thus, melanocortin MC_4 receptor activation is sufficient for erectogenesis and these effects may involve oxytocinergic pathways.

Keywords: Erectile dysfunction; Melanocortin; Melanocortin MC_4 receptor; G protein-coupled receptor

1. Introduction

The melanocortin peptides adrenocorticotropic hormone (ACTH), α-, β- and γ-melanocyte stimulating hormone (MSH) exert a host of diverse physiological effects in the periphery and in the central nervous system (CNS) (Wikberg et al., 2000). The specificity of these pleiotropic peptides is conferred by both tissue-specific post-translational processing of the pro-opiomelanocortin (POMC) precursor protein, which yields distinct peptides, and by the selective interactions of these peptides with one of the five cloned melanocortin receptors. The distribution of these receptors, like that of POMC, is widespread throughout the body. To the extent that expression of a given peptide and a receptor subtype overlap, the function of each within a given tissue is clear. For example, in the periphery, the effects of α-melanocyte stimulating hormone (α-MSH) on melanocytes and of ACTH on the adrenal gland are mediated by melanocortin MC_1 and melanocortin MC_2 receptors, respectively (Wikberg et al., 2000). By contrast, in the CNS where melanocortin MC_3 and MC_5 receptors are often expressed in overlapping brain regions, it is difficult to ascribe the actions of α-MSH and ACTH to a single receptor subtype since each peptide fails to discriminate among the melanocortin receptors (Adan and Gispen, 2000).

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Central administration of α-MSH and ACTH elicits a range of behavioral effects including a syndrome consisting of stretching and yawning which is often associated with penile erection (Gessa et al., 1967; Bertolini and Gessa, 1981; Vergoni et al., 1998). Since the original descriptions of this syndrome, numerous studies have sought to identify the neural substrates and, more recently, the anatomical regions and receptors through which these effects are mediated. To this end, Argiolas et al. (2000) demonstrated that microinjections of ACTH and α-MSH into specific hypothalamic regions, including the paraventricular nucleus reproduce the behavioral syndrome observed with intracerebroventricular (i.c.v.) injections of ACTH and α-MSH.

While stretching and yawning are often associated with penile erection, these behaviors need not necessarily occur together (Bertolini et al., 1969; Bertolini and Gessa, 1981) and may be pharmacologically distinct. Administration of a melanocortin MC_4 receptor preferring antagonist (Schioth et al., 1998), HS014, blocks stretching and yawning behavior, but not the penile erections produced by ACTH and α-MSH (Vergoni et al., 1998; Argiolas et al., 2000) and does not influence sexual behavior in male rats (Vergoni et al., 2000). These results illustrate the independence of the stretching/yawning—penile erection responses and further were interpreted to indicate that the pro-erectile effects of non-subtype selective melanocortin agonists are mediated by melanocortin MC_3, not melanocortin MC_4, receptors. However, these findings contrast with our recently described results in which a selective melanocortin MC_4 receptor agonist enhances electrical stimulation-evoked increases in intracavernosal pressure and facilitates sexual behavior in mice. Furthermore, mice lacking melanocortin MC_4 receptor failed to respond to the agonist and exhibited compromised sexual function (Van der Ploeg et al., 2002). The importance of defining the relative contributions of melanocortin MC_3 and MC_4 receptors to erectile function is underscored by the reports that melanotan-II, a synthetic α-MSH analog induces penile erections in humans (Wessells et al., 1998, 2000).

One of the unique features of melanotan-II, compared with α-MSH, is that it induces pro-erectile effects at very low doses (0.025 mg/kg, s.c.) following systemic administration. Since melanotan-II, like its endogenous counterpart, does not discriminate amongst melanocortin receptors, we sought to further characterize the involvement of melanocortin MC_4 receptor in erectogenesis using rodent models of erectile function. Penile erections can be evoked in rats, out of the context of copulation (*ex copula*), by retracting the penile sheath (Sachs, 2000). This retraction applies tonic pressure at the base of the glans that can trigger clusters of erections, mediated by a reflexogenic arc comprised of the penis, spinal cord and peripheral nerves, which are modulated by peripheral and descending supraspinal input (Andersson and Wagner, 1995). Erections occurring *ex copula* are associated with, and presumably mediated by, changes in intracavernosal pressure. Thus, by studying penile erections and changes in intracavernosal pressure *ex copula*, one can evaluate putative erectogenic agents that may enhance erectile activity triggered by activation of an erectogenic reflex loop via receptors at several levels of the neuraxis.

2. Materials and methods

2.1. Ex copula conditioning and reflex tests

All experiments were approved by the Merck Research Laboratories-Rahway Institutional Animal Care and Use Committee and conform to the European Community guidelines for the use of experimental animals. Sexually mature male Sprague–Dawley rats (Charles River 300–500 g) with the suspensory ligament removed were conditioned over 5 days prior to quantification of penile erections (test days 1 and 2). On day 1 of conditioning, animals were placed in the supine position with their anterior torso placed inside a darkened plastic 8-cm cylinder for 30 min. The lower torso and hind limbs were restrained with nonadhesive material (Vetrap, 3M, Henry Schein, Melville, NY). On days 2, 3, and 4, the animals were restrained in the supine position for 30 min and the glans penis was passed through an additional piece of Vetrap which was fastened over the animal to maintain the preputial sheath in a retracted position. On day 5, the day 1 procedure was repeated.

Penile responses, typically termed *ex copula* genital reflex tests (Sachs, 2000), were quantified following vehicle and drug treatment on two different days. On the first test day, rats received vehicle treatment, were restrained in the supine position with the preputial sheath retracted and the number of penile responses were videotaped for 20 min after the first erection occurred. After 1 day of rest, these same rats received drug treatment and were evaluated for penile responses as described above. Typically, a series of penile erections occurred spontaneously within a few minutes after sheath retraction. Normal reflexogenic erectile responses which were counted as erections included elongation (an extension of the penile body) and engorgement (dilation of the glans penis). "Cups" (an intense erection where the distal margin of the glans penis momentarily flares open to form a cup) and "flips" (a dorsiflexion of the penile body) were noted, but not quantified. Videotapes were read by an observer blinded to the treatment condition and the total number of erections was counted for 15 min after the first response. Percent change from vehicle was calculated. Results are expressed as mean ± standard error of mean. Statistical significance was determined by a paired Student's *t*-test where a $P < 0.05$ was considered significant. At the conclusion of the experiments, animals were euthanized by CO_2 asphyxiation.

2.2. Telemetric recording of intracavernosal pressure

TA11PA-C40 pressure transducers (8 cm catheter line and 3 mm gel tip, Data Sciences International, St. Paul, MN) were implanted in sexually mature male Sprague–Dawley CD rats (300–400 g; Charles River, Wilmington, MA) under ketamine (87 mg/kg, i.p., Animal Health, Fort Dodge, IA) xylazine (13 mg/kg, i.p., Bayer, Shawnee Mission, KS) anesthetic cocktail. Rats were placed in dorsal recumbency and the region from the abdomen to the scrotum was shaved and surgically prepared. With the penis retracted, a 2–3-cm vertical incision was made in the mid-proximal dermal layer of the scrotum. The penis body was bluntly exposed and a transmitter tunnel was formed subcutaneously from the scrotum to the lower right abdomen in which the unit body was implanted. The transducer line was placed 2–3 mm deep in the lateral portion of the proximal cavernosum with a 23-gauge butterfly needle line filled with saline. Proper placement was confirmed by an injection of saline that inflated the cavernous space. The butterfly needle was slowly removed and a bent-beveled 20-gauge needle or plastic needle was inserted into the puncture site to introduce the transducer tip 2–4 mm into the cavernosum. The introducer was removed and the cannula was fixed and the puncture closed with a single drop of 3M Vetbond™ tissue adhesive. One or two very loose sutures (6.0 VICRYL, P-1) were placed in cavernosum/fascial tissue to secure the transducer line below the catheter entry site. Subcutaneous tissue was closed with a continuous suture and the skin incision was closed with 2–3 Clay Adams 9-mm stainless steel staples. Each rat received Children's Tylenol® Elixir (48 mg, p.o.) perioperatively, was treated with 45,000 U Pen-G[3]antibiotic and allowed to recover for a minimum of 5 days. Animals were given free access to food and water and individually housed in Nalgene® rat cages in a room a 12:12-h light cycle (7 AM ON).

Each animal's telemetry signal was calibrated and evaluated for stable baselines during both sleep and active behaviors. Appropriate pressure increases during gentle manipulation were performed as validation that the pressure transducers remained viable. Sixty to eighty percent of the rats exhibited telemetry signals during rest, activity and stimulation which were judged to be suitable for inclusion in the experimental protocol. Rats were dosed in a two period crossover design in which half the animals were randomly selected for drug treatment (THIQ 1 mg/kg, i.v.; $n = 14$) and the remaining received vehicle (10% ethanol, 40% polyethylene glycol (PEG)400, 50% saline) on the first day. On the second day, the opposite treatment was given to each animal. Dosing occurred between 10 AM and 2 PM. After dosing, each animal was positioned for *ex copula* evaluation with penile sheath retracted, and placed on their receiver. Data were collected for ~ 60 min (40-min post dose).

Data were received by a Data Sciences International A. R. T. Analog 8 Source system using DSI telemetry software (St. Paul, MN) and were acquired at 100 Hz on an auxiliary computer configured with custom designed software from Foxglove Systems (Morristown, NJ). The data from each animal was displayed graphically and a threshold set based on baseline (flaccid) intracavernous pressure ± 2 S.E.M. determined as the mean pressure during a 5-min period. In practice, this threshold was sufficiently high to discriminate penile erection from common noise. Intracavernous pressure that exceeded the threshold for 15 s or more was defined as an interval. Intracavernous pressure spikes of less than 15 s in duration that were >30 mm Hg above the threshold were defined as single events. Erectile activity was calculated from the total number of intervals and single events. For intervals, the magnitude (area under the curve) and duration of each interval were also determined. The effect of treatment was calculated by comparing the single events, intervals, area under the curve and duration in vehicle and drug treated rats. Analysis of variance (ANOVA), followed by post hoc analyses or two-tailed Student's *t*-tests, where appropriate, were used to determine statistical significance.

2.3. Intracerebroventricular administration

For intracerebroventricular (i.c.v.) dosing, sexually mature male rats (as described above) were obtained from Charles River Laboratories with cannulae implanted into the 3rd ventricle. Cannula placement was confirmed by i.c.v. administration of angiotensin II (20 ng/0.4 μl) which induces thirst and stimulates water consumption. Rats that consumed 5 ml of water within 30 min of angiotensin II dosing were judged to have properly located, functional cannulae and were included in subsequent studies following a 4-day wash out period.

2.4. Chemicals

Apomorphine (Sigma, St. Louis, MO) was dissolved in saline with 0.1% Ascorbic Acid and was dosed in a volume of 0.5 ml/kg, s.c. *N*-[(3*R*)-1,2,3,4-tetrahydroisoquinolinium-3-ylcarbonyl]-(1*R*)-1-(4-chlorobenzyl)-2-[4-cyclohexyl-4-(1*H*-1,2,4-triazol-1-ylmethyl)piperidin-1-yl]-2-oxoethylamine (THIQ; Van der Ploeg et al., 2002), sildenafil (Merck-Frosst, Montreal) and an oxytocin antagonist, L-368899 (Williams et al., 1994) were dissolved in 10% ethanol, 40% PEG400, 50% saline and were administered in a volume of 0.5 ml/kg. For i.c.v. administration, agouti-related protein (AgRP-83-132; Phoenix, Belmont, CA) and a nonselective endogenous melanocortin MC_4 receptor preferring antagonist, MBP10 (Bednarek et al., 2001) were dissolved in artificial cerebral spinal fluid (CSF) and administered in a total volume of 0.4 μl. L-368899 and THIQ were dissolved in propylene glycol (50% and 66%, respectively) and artificial CSF and administered in a total volume of 0.6 μl. THIQ has moderate bioavailability (14%), is readily absorbed ($T_{max} = 1$ h) and has a relatively short $T_{1/2}$ (0.5 h; Sebhat et al., in press).

3. Results

3.1. Telemetric monitoring of intracavernous pressure ex copula

Fig. 1 illustrates the relationship between intracavernous pressure recorded telemetrically and erectile activity determined visually in the *ex copula* model. In this example, the duration of this erectile episode was 46.7 s. During this interval, three erections occurred, each at different magnitudes of change in intracavernous pressure. The first erection was not observed until intracavernous pressure was elevated for approximately 15 s. Thus, both the magnitude of intracavernous pressure and the time it remains elevated determine the nature of the penile response.

We observed significant increases in intracavernous pressure over the 40-min recording period regardless of treatment and notable differences between vehicle and THIQ-treated rats (Fig. 2A–B). The melanocortin MC_4 receptor agonist markedly enhanced the number of episodes in which intracavernous pressure remained elevated above threshold for longer than 15 s (intervals). Seventy-one percent of THIQ-treated rats (10/14) exhibited more than five intervals during the 40 min following treatment, compared with only 43% that received vehicle (6/14). Although there was no statistical difference in the total number of intervals (Fig. 2C), the magnitude ($F(8,208)=2.75$, $P=0.007$; Fig. 2D) and the duration ($F(8,208)=3.80$, $P=0.003$; Fig. 1E) of intervals were significantly greater in THIQ-treated rats. Furthermore, THIQ prolonged the mean duration of each interval by ca. 15 s (43.0 ± 6.8 vs. 28.4 ± 3.8 s; $P<0.05$, paired *t*-test). By contrast, THIQ did not affect the transient increases in intracavernous pressure, those lasting less than 15 s, compared with vehicle (data not shown).

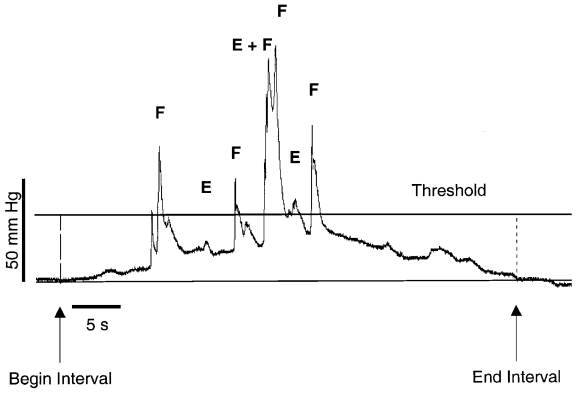

Fig. 1. Relationship between intracavernous pressure recorded telemetrically in awake rats and erectile activity determined visually. Increases in intracavernous pressure were considered significant when intracavernous pressure exceed flaccid intracavernous pressure ±2 S.E.M. Increases of <15 s were classified as single events; whereas episodes >15 s were considered intervals (marked by arrows). The start and end to an interval was determined retrospectively, only after threshold had been exceeded for >15 s. In this example, the rat was videotaped so that penile erections could be quantified and compared to changes in intracavernous pressure. 'E' denotes an erection; 'F' indicates a flip. See Sections 2.1 and 3.1 for details.

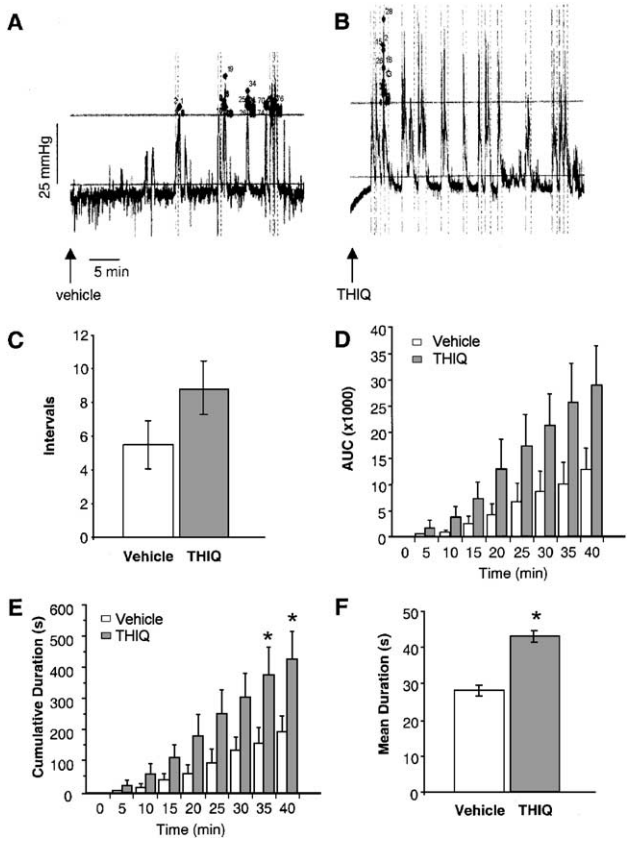

Fig. 2. Intracavernous pressure recorded telemetrically in awake rats. Individual examples of the effects of (A) vehicle and (B) THIQ (1 mg/kg, i.v.) on intracavernous pressure activity. Based on flaccid intracavernous pressure (lower horizontal line), a threshold (higher horizontal line) was set to detect significant increases in intracavernous pressure. Vertical lines represent erectile episodes that last >15 s. The magnitude (C and D) of these episodes were quantified over 40 min for each treatment ($n=14$). Effect of vehicle and THIQ (1 mg/kg, i.v.) on the (E) number and (F) duration of erectile episodes. *$P<0.05$; see Sections 2.1 and 3.1 for details.

3.2. Penile erections ex copula

In the *ex copula* model, vehicle treated rats exhibited on average 24.0 ± 1.3 penile erections within a 15-min time window (range = 12.1–35.1). We examined the reproducibility of the penile reflexes and found that repeated administration of vehicle (1 day between injections) alone did not influence the number of penile erections (24.3 ± 2.2 vs. 25.7 ± 3.3, $P>0.05$; Fig. 3A). Systemic administration of THIQ significantly increased the number of penile erections at 1 and 5 mg/kg, i.v. by 37% and 92%, respectively ($P<0.001$, Fig. 3A). In a series of seven separate experiments, THIQ (1 mg/kg, i.v.) produced statistically significant increases in erectile responses on six occasions with a mean increase of $58 \pm 7\%$ ($n=66$, Fig. 3B). At 10 mg/kg,

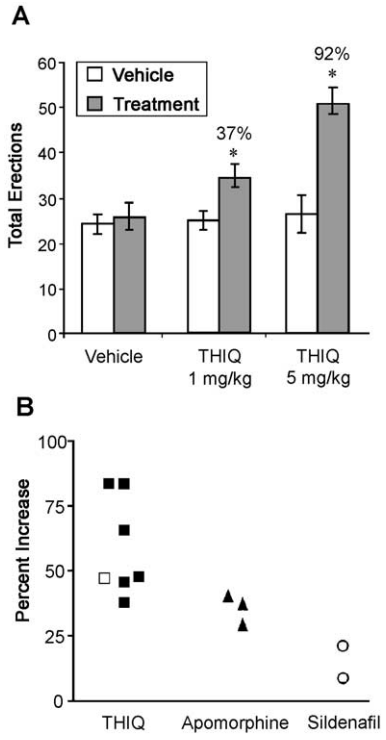

Fig. 3. Penile erections in *ex copula* model. (A) THIQ dose-dependently (1 and 5 mg/kg, i.v.) increased the number of penile erections. Values represent mean ± S.E.M. *$P<0.05$, paired *t*-test. (B) Comparison of THIQ (squares, 1 mg/kg, iv, n = 66), apomorphine (triangles, 0.025 mg/kg, s.c., $n=30$) and sildenafil (circles, 1 mg/kg, i.v., $n=25$). Each symbol represents a single experiment of $n=5-10$ rats per experiment. Filled symbols indicate $P<0.05$; open symbols indicate $P>0.05$.

i.v., THIQ failed to significantly enhance yawning, stretching or genital grooming behavior ($n=6-10$/group; data not shown). Although the magnitude of erectogenesis produced by THIQ at 1 mg/kg, i.v. varied from 37% to 83% across experiments, it compared favorably with that of apomorphine (0.025 mg/kg, s.c.) which consistently increased penile erections by 35 ± 3% in three separate experiments ($n=30$). Moreover, higher doses of apomorphine (0.05–0.1 mg/kg, s.c.) did not further increase erectile responses (data not shown). Unlike THIQ and apomorphine, sildenafil (1 mg/kg, i.v.) failed to increase penile erections (13 ± 10%, $P>0.05$; Fig. 3B). In addition to the number of erections produced by the putative erectogenic agents, we also examined the time required to elicit the first penile erection. The mean onset following vehicle administration was 14.3 ± 2.9 min. THIQ did not significantly decrease the latency to the first erectile event at either 1 (2%) or 5 mg/kg (32%); whereas apomorphine decreased onset by 70%, to ca. 4 min ($P<0.05$; Fig. 4). Consistent with a lack of effect on the number of erections, sildenafil treatment (1 mg/kg, i.v.)

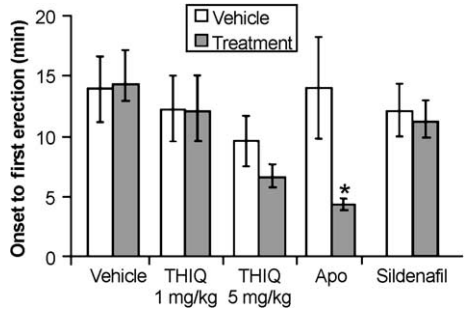

Fig. 4. Time to first penile erection following vehicle, THIQ, apomorphine and sildenafil. *$P<0.05$. Values represent mean ± S.E.M.

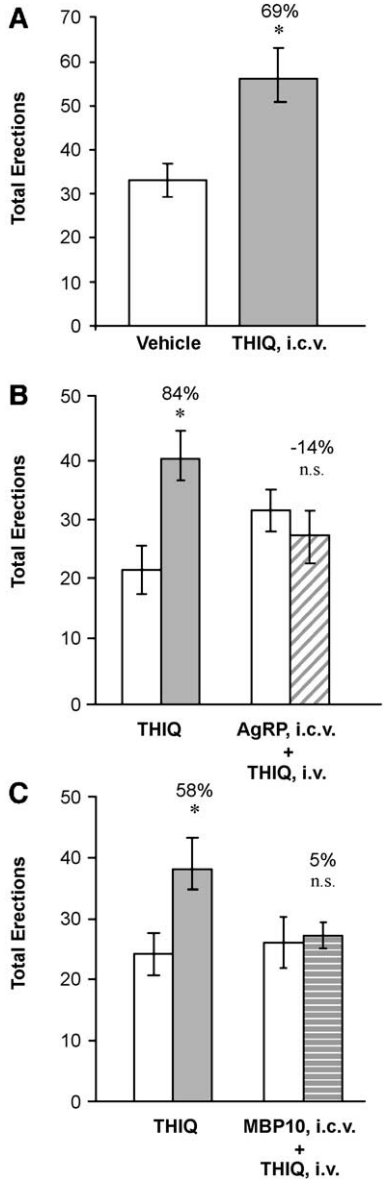

Fig. 5. Effect of centrally administered (i.c.v.) (A) THIQ (20.0 μg, $n=6$) on penile erections and (B) a nonselective melanocortin receptor antagonist (AgRP, 5.5 μg, $n=7$) and (C) selective melanocortin MC_4 receptor antagonist (MBP10, 5.5 μg, $n=16$) on penile erections induced by THIQ (1 mg/kg, i.v.). *$P<0.05$, paired *t*-test. Values represent mean ± S.E.M.

did not affect the latency to the first erectile response (8%, $P>0.05$).

Since penile reflexes elicited by mechanical stimulation (retraction of the penile sheath) need not activate the same pro-erectile pathways through which centrally administered melanocortin peptides produce their effects, we tested whether direct (i.c.v.) injection of THIQ would affect erectile activity. THIQ (20.0 μg) significantly increased the number of penile erections by 69% after central administration ($P<0.05$, paired t-test; Fig. 5A). Furthermore, i.c.v. administration of agouti-related protein (AgRP, 5.5 μg), a competitive non-subtype selective endogenous melanocortin receptor antagonist, did not significantly reduce the number of penile erections (not shown), but completely blocked the pro-erectile effects of systemically administered THIQ (Fig. 5B). Consistent with these results, systemic administration of an melanocortin MC_4 receptor selective antagonist, MBP10 (1 mg/kg, i.v. (Bednarek et al., 2001), also antagonized the effects of 1 mg/kg, i.v. THIQ (Fig. 5C). Furthermore, systemic administration (1 mg/kg, i.v.) of a selective oxytocin antagonist, L-368899, attenuated the pro-erectile effects of THIQ from 47% to 21% ($P>0.05$; Fig. 6A), but did not significantly influence the number of erections when administered alone (4%, data not shown). Moreover, central administration of L-368899 (5.0 μg) completely blocked THIQ-induced penile erections (from 46% to −11%; $P>0.05$; Fig. 6B).

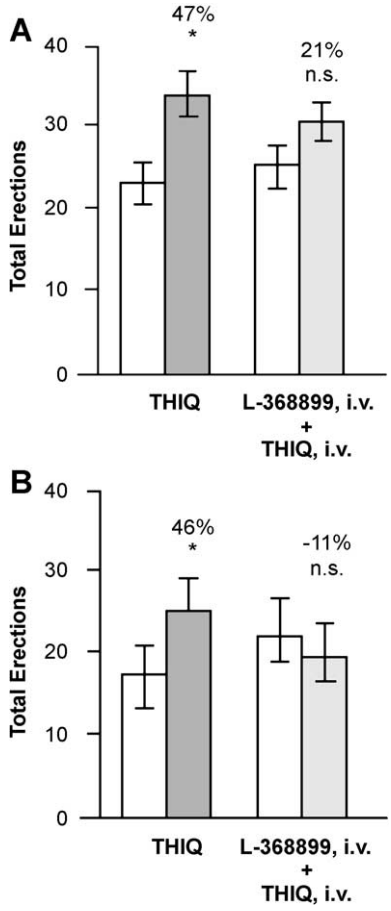

Fig. 6. Effect of an oxytocin receptor antagonist, L-368899 administered (A) systemically (1 mg/kg, i.v., $n=20$) and (B) centrally (5.0 μg, $n=10$) on erectogenic effects of THIQ (1 mg/kg, i.v., $n=10$). *$P<0.05$, paired t-test. Values represent mean ± S.E.M.

4. Discussion

In these studies, we demonstrated that activation of melanocortin MC_4 receptor by a potent agonist, with high selectivity over the other melanocortin receptors as well as over other receptors, ion channels and enzymes, is sufficient to enhance intracavernous pressure and the number of penile erections in conscious rats outside of a sexual context. We found that in the *ex copula* model male rats present with marked erectile activity, determined by both increases in intracavernous pressure and penile erections. Systemic administration of THIQ augmented these increases in intracavernous pressure and enhanced the number of penile erections over the 15-min observation period. Pro-erectile effects were elicited by both central and systemic administration of THIQ. Since central injection of the endogenous non-subtype specific melanocortin receptor antagonist AgRP and intravenous injection of MBP10, a melanocortin MC_4 receptor preferring antagonist blocks the penile erections induced by intravenous THIQ, melanocortin MC_4 receptors likely mediate the effects of THIQ. Moreover, we showed that melanocortin MC_4 receptor-mediated erectogenesis requires the activation of central oxytocinergic pathways that modulate erectile activity.

Telemetric recording of intracavernous pressure in awake rats provides a means of quantitatively assessing erectile function (Bernabe et al., 1999) and is useful for examining the erectogenic effects of pharmacological agents (Andersson et al., 1999). THIQ did not reduce the time to observe significant increases in intracavernous pressure or the number of transient rises in intracavernous pressure (<15 s). However, THIQ tended to increase the magnitude of these responses as well as the number of episodes in which intracavernous pressure remained above a pre-determined threshold for more than 15 s (intervals), a requirement for a penile erection. The duration of each interval recorded following THIQ treatment was significantly longer than that observed after vehicle administration.

We showed, as others have (Bernabe et al., 1995, 1999), that penile erections in awake rats are associated with meaningful increases in intracavernosal pressure. Although telemetric recording of intracavernous pressure provides an effective means for detecting erectile activity, as it is conducted in conscious animals, it is subject to extraneous stimuli producing variability. Moreover, the assay is labor intensive requiring conditioning, visual evaluation and scoring of each subject. Since penile erection in rats can be evoked by retracting the penile sheath, we used this method

to understand the contribution of melanocortin MC_4 receptor to this phenomenon. We minimized the possibility that presumed pro-erectile effects were merely the result of inter-animal variability by using a within animal experimental design in which the effects of treatment were determined relative to the vehicle administration. It is also unlikely that testing alone accounted for the increase in penile erections we observed following treatment since repeated vehicle treatments did not increase the total number of erections.

In agreement with our findings with intracavernous pressure, THIQ consistently increased the total number of penile erections; whereas the time of onset to the first erection was unchanged. This profile is distinct from that of apomorphine, a known erectogenic agent (Andersson, 2001), which decreased the latency to the first erection and increased the number of erections and from sildenafil which affected neither the onset nor the total number of erections.

These results confirm and extend some previously reported findings with α-MSH, namely that melanocortin receptors modulate erectile activity and that melanocortin MC_4 receptors participate in this modulation. Numerous reports have demonstrated that i.c.v. and intracerebral administration of the non-subtype selective peptides ACTH and α-MSH stimulates penile erections (Argiolas, 1999; Argiolas et al., 2000; Bertolini and Gessa, 1981; Bertolini et al., 1969; Mizusawa et al., 2002). Our results in which i.c.v. administration of THIQ increases penile erections and the central administration of the nonselective endogenous melanocortin receptor antagonist AgRP blocks the effects of systemically administered THIQ confirm the involvement of brain melanocortin receptors in penile erection. That activation of melanocortin MC_4 receptors is required for erectogenesis is strengthened by our finding that MBP10, a melanocortin MC_4 receptor selective peptide antagonist, also attenuated the number of penile erections induced by systemic administration of THIQ.

Our findings here suggest that melanocortin MC_4 receptor activation is sufficient for pro-erectile activity. This interpretation is supported by the demonstration THIQ (10 mg/kg, i.v.) augmented intracavernosal pressure evoked by electrical stimulation of the cavernous nerve in wild-type, but not melanocortin MC_4 receptor knock-out mice (Van der Ploeg et al., 2002), but, conflicts with other reports in which melanocortin MC_4 receptor antagonists were evaluated. Intracerebroventricular or intra-hypothalamic injection of a melanocortin MC_4 receptor specific antagonist, HS014, failed to block α-MSH-induced penile erections at doses that inhibited stretching and yawning (Argiolas et al., 2000; Vergoni et al., 1998). This result implicates melanocortin MC_3, not melanocortin MC_4, receptors, in the penile erections induced by non-subtype selective melanocortin receptor agonists. Although HS014 is a potent antagonist at melanocortin MC_4 receptors, it exhibits modest selectivity for melanocortin MC_4 receptors over melanocortin MC_3 receptors (~ 17-fold; Bednarek et al., 2001; Schioth et al., 1998) compared with MBP10 (300-fold; Bednarek et al., 2001). Nonetheless, it is difficult to reconcile how HS014 blocks stretching, yawning and grooming behavior, but not penile erections, especially in light of the postulated role of melanocortin MC_4 receptors in grooming behavior (Adan et al., 1999). One possibility is that ACTH and α-MSH-induced penile erections are mediated by both melanocortin MC_3 and melanocortin MC_4 receptors, both of which are expressed in regions of the brain (Roselli-Rehfuss et al., 1993; Mountjoy et al., 1994) that modulate erectile and sexual behavior (McKenna, 2000). Indeed, SHU 9119, a nonselective antagonist at melanocortin MC_3 and melanocortin MC_4 receptors, blocked melanotan-II induced increases in intracavernous pressure in anesthetized rabbits (Vemulapalli et al., 2001).

Since THIQ is >3000-fold more selective for melanocortin MC_4 receptor over melanocortin MC_3 receptor (Van der Ploeg et al., 2002), selective blockade of melanocortin MC_4 receptors by MBP10 would be expected to be sufficient to attenuate the pro-erectile effects, as we demonstrated in this study. However, it is worth noting that the doses of THIQ required to increase penile erection *ex copula* in this study are significantly higher than the doses of melanotan-II that stimulate erections in man. The higher dose requirement for the melanocortin MC_4-preferring agonist, THIQ, to elicit pro-erectile activity, relative to melanotan-II, may be attributable to its poor brain penetration (brain-to-plasma ratio = 0.1) and/or high plasma protein binding (>95%; unpublished observations) or lower intrinsic potency of THIQ at the melanocortin MC_4 receptor (cf. Adan et al., 1999; Van der Ploeg et al., 2002). Although our and previous (Van der Ploeg et al., 2002) data suggest that activation of melanocortin MC_4 receptor is required for the pro-erectile effects of melanocortins, we cannot exclude the possibility that concomitant activation of melanocortin $MC_{3,4}$ and MC_5 receptors increases erectile activity more than activation of the melanocortin MC_4 subtype alone. Likewise, the lack of statistically significant changes in stretching and yawning behavior, at least after systemic administration of THIQ, suggests that activation of melanocortin MC_3 receptors and/or greater activation of brain melanocortin MC_4 receptors may be necessary for this behavioral syndrome. Oxytocin plays an essential role in the expression of penile erection and copulatory behavior. Oxytocin receptor antagonists inhibit not only penile erections induced by oxytocin but also those induced by sexual stimuli or other drugs, such as dopamine receptor agonists (see Argiolas, 1999 for review). We found that systemic and central administration of an oxytocin receptor antagonist, L-368899, significantly reduced the number of penile erections produced by THIQ (1 mg/kg, i.v.). This result illustrates the importance of oxytocinergic pathways in the melanocortin MC_4 receptor-mediated erectogenesis. However, Mizusawa et al. (2002) reported that i.c.v. administration of α-MSH induced penile erections and increased intracavernous pressure in anesthetized rats, but that these effects were not blocked by an oxytocin antagonist, l-de-

amino, 2-D-Tyr(Oet), 4-Thr, 8-Orn-OT. Similarly, d(CH2)5-Tyr(Me)-[Orn8]vasotocin, a potent oxytocin antagonist (10 μg, i.c.v.), also failed to reduce penile erections produced by ACTH (Argiolas et al., 1987). Whether the differential use of THIQ vs. α-MSH accounts for this apparent discrepancy remains to be determined.

Previous work has shown that neither melanotan-II (Vemulapalli et al., 2001), nor a melanocortin MC_4 receptor agonist (Van der Ploeg et al., 2002) exerts direct actions on the corpus cavernosum which suggests that central pathways within the brain and spinal cord mediate melanocortin-induced erectogenesis. By studying penile erections and changes in intracavernous pressure that are triggered by activation of an erectogenic reflex loop which is under the control of peripheral and descending supraspinal input, we have shown that systemic administration of a melanocortin MC_4 receptor agonist increases the number of erections observed *ex copula*. This is achieved by increasing the time during which intracavernous pressure remains elevated above a threshold level. Like previous studies in which non-subtype selective melanocortin receptor peptide agonists, such as α-MSH, were injected directly into the brain, we show that central administration of THIQ increases penile erections. Importantly, intravenous administration of a melanocortin MC_4 receptor preferring agonist, which permits the activation of melanocortin MC_4 receptors within central and peripheral pathways, also influence erectile activity. Similarly, the inhibition of THIQ-induced erectogenesis by systemic and central administration of an oxytocin antagonist suggests that oxytocin release within these pathways may be essential for melanocortin MC_4 receptor-mediated enhancement of erectile activity. Melanocortin MC_4 receptor mRNA has been localized to regions within the brain, spinal cord and mechanosensory afferents that influence erectile activity (Roselli-Rehfuss et al., 1993; Mountjoy et al., 1994; Van der Ploeg et al., 2002). Our results suggest that modulation of these networks via melanocortin MC_4 receptor activation may account for the pro-erectile effects observed with systemic administration of melanotan-II in man.

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