

Control of Penile Erection by the Melanocortinergic System: Experimental Evidences and Therapeutic Perspectives

Review

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Penile erection is the ultimate vasculo-tissular response to an integrated and complex series of physiological processes elicited by psychological, neural, and hemodynamic events (Giuliano and Rampin, 2000; Saenz de Tejada, 2002). Upon sexual stimulation, penile erection, occurring in response to the activation of proerectile autonomic pathways, is greatly dependent on adequate inflow of blood to the erectile tissue and requires coordinated arterial endothelium-dependent vasodilatation and sinusoidal endothelium-dependent corporal smooth muscle relaxation.

Most of the research efforts toward the treatment of erectile dysfunction (ED) have targeted the peripheral mechanisms of penile erection. These efforts have led to the identification of several compounds that can induce smooth muscle relaxation and erection by acting on cellular receptors (eg, prostaglandin E₁) (Hedlund and Andersson, 1985; Stackl et al, 1988) or the intracellular machinery involved in the transduction of the signal (eg, sildenafil and other phosphodiesterase 5 inhibitors) (Giuliano, 2002). The registration in several countries of apomorphine for the treatment of ED has demonstrated the possibility of enhancing penile erection by acting elsewhere than on corpus cavernosum smooth muscle cells (Giuliano and Allard, 2002).

The central nervous system (CNS) and, more specifically, the structures and neurotransmitters involved in the control of penile erection, represent, at least theoretically, a valuable alternative target for overcoming ED. In the CNS, several effectors involved in the control of erectile function have been identified. These include primarily dopamine, serotonin, noradrenaline, excitatory amino acids,

oxytocin, and nitric oxide, among others (Moreland et al, 2000; Andersson, 2001). The present review is focused on the role of the melanocortinergic system in the regulation of penile erection. Several lines of evidence suggest that the melanocortinergic system may represent a potential valid pharmacological target for the treatment of ED. Because the interaction between the melanocortinergic system and erection seems to be neurally mediated, the review first briefly summarizes the role of the CNS in the command of erection.

Neural Control of Penile Erection

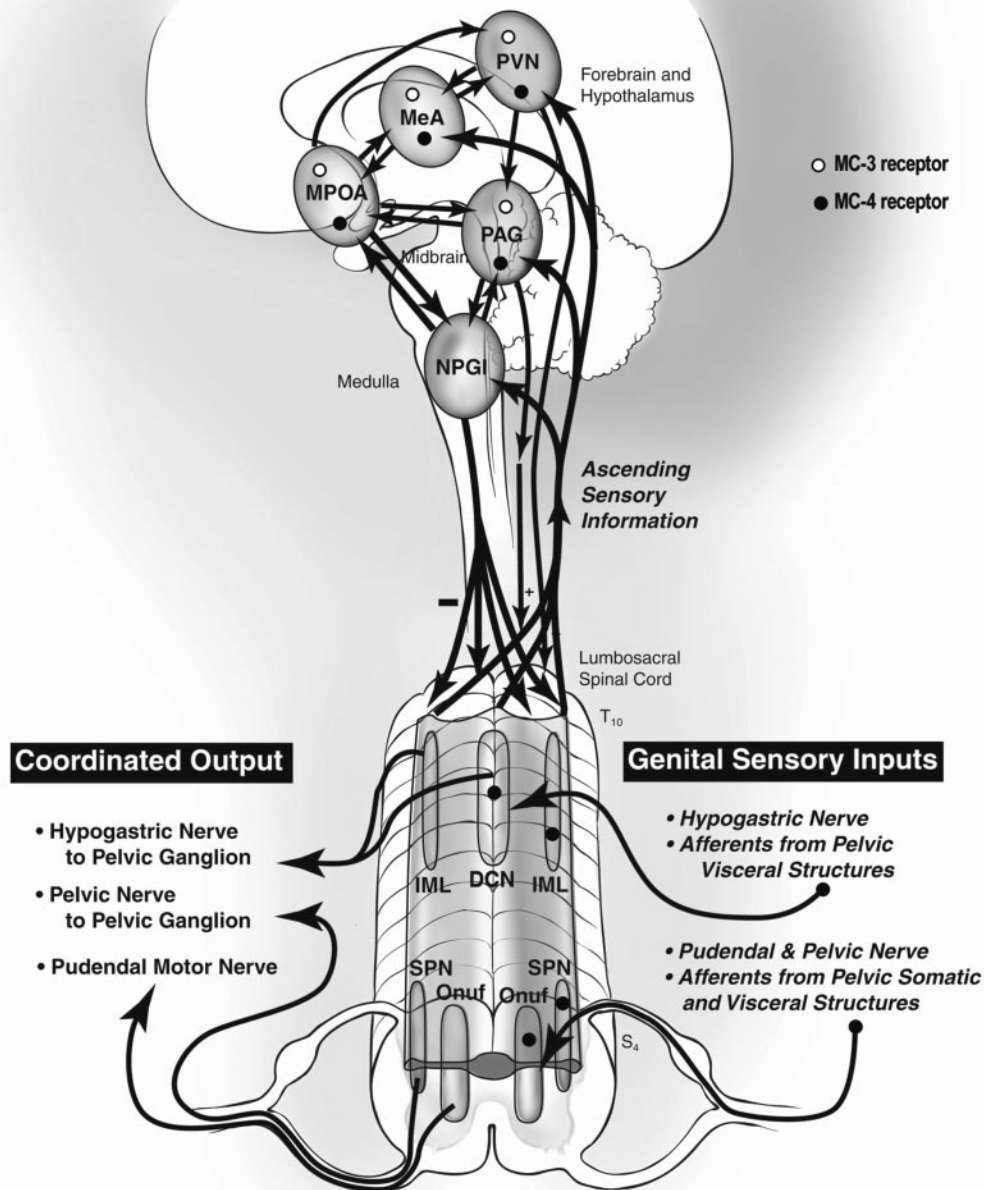
Sacral parasympathetic outflow, emanating from the intermediolateral cell column of the S2 to S4 spinal segments, is the proerectile pathway (Giuliano et al, 1995) (Figure). Sympathetic innervation of the pelvis, issuing from spinal segments T11 to L2, is responsible for the basal antierecile tone present during the flaccid state (Giuliano et al, 1997). Thus, it may be assumed that inhibition of the sympathetic component occurs during penile erection. Additionally, contraction of the ischiocavernosus and bulbospongiosus striated muscles, located at the penile crus and innervated by the motor pudendal nerve with cell bodies of motoneurons located in the Onuf nucleus, has a definite, contributory role in penile erection (Schmidt and Schmidt, 1993). Accordingly, the spinal coordination of various autonomic and somatic nuclei is required for a rigid and sustained penile erection (Rampin et al, 1997).

The penis is also considered to be a sensory organ that sends information to the CNS via afferents. Indeed, the spinal autonomic nuclei controlling penile erection receive afferent information conveyed by afferent sensory fibers that originate from the penis and perigenital area, which are conveyed mainly by the dorsal nerve of the penis.

The CNS maintains primary control over sexual motivation and penile erection (Giuliano et al, 2000). The precise sequence of neurotransmitter release within the CNS leading to penile erection is unknown. In the limbic system and, notably, in the nucleus accumbens, dopamine plays a key role in the anticipatory phase of sexual behavior. Participation of the noradrenergic system is also likely in the motivational aspect of sexual behavior. The medial preoptic area in the hypothalamus is essential for the display of sexual behavior, likely by integrating and redistributing information to hypothalamic and brainstem

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Organization of the central and peripheral nervous systems involved in the control of ED. The localization of MC-3 and MC-4 receptors at different levels is represented. DCN indicates dorsal column nucleus; IML, intermediolateral cell column; MeA, medial amygdala; MPOA, medial preoptic area; PAG, periaqueductal gray; PVN, paraventricular nucleus; SPN, sympathetic preganglionic neurons; and NPGi, nucleus paragigantocellularis. Adapted, with permission, from McKenna (1999).

structures, such as the paraventricular nucleus (PVN) of the hypothalamus and the nucleus paragigantocellularis (nPGi) in the pons. In penile erection, dopamine activates oxytocinergic neurons in the PVN. Serotonergic projec-

tions from the nPGi to the SPN likely exert a tonic inhibition on erectile response, which are likely abolished upon sexual arousal by projections from the PVN to the nPGi.

*Melanocortin receptor expression and function**

	MC-1 Receptor	MC-2 Receptor	MC-3 Receptor	MC-4 Receptor	MC-5 Receptor
Expression	Melanoma cells, melanocytes, skin gland, hair follicle, testis, pituitary, anti-inflammatory cells, periaqueductal gray	Adrenal cortex, adipocytes, skin	Brain, placenta, gut, heart, testis	Brain, adipose tissue	Adrenal gland, adipose tissue, kidney, leukocytes, lung, lymph node, mammary gland, ovary, testis, uterus, brain, skeletal muscle, exocrine tissues
Function	Pigmentation, inflammation	Steroidogenesis	Energy homeostasis, sexual behavior (?)	Appetitive regulation, sexual behavior	Exocrine function

* Adapted from Voisey (2003).

Melanocortin System

Melanocortin Peptides—Melanocortins are bioactive peptides that are widely expressed in the CNS and in various peripheral tissues. These peptides are involved in the regulation of important physiological functions including food intake, energy homeostasis, and immune function. The discovery of the contribution of melanocortin peptides in the regulation of sexual function aroused new interest in the complex function of these peptides (Wikberg et al, 2000). The melanocortins comprise a group of natural peptides, all of which are derived from the precursor molecule proopiomelanocortin (POMC). POMC is a polyhormone that can give rise to at least 8 distinct peptides whose biologic roles are incompletely delineated (Hadley and Haskell-Luevano, 1999). Cleavage at tetra-basic sites is an important regulatory step in the processing of POMC in the pituitary, where tissue-specific cleavage at the LysLysArgArg site in POMC produces either adrenocorticotropin (ACTH) in the anterior pituitary, or alpha-melanocyte-stimulating hormone (α -MSH) in the intermediate pituitary. Historically, POMC was thought to be produced solely by pituitary cells, but it has become apparent that POMC messenger RNA (mRNA) or POMC-derived peptides are expressed in extrapituitary tissues, such as the arcuate nucleus of the hypothalamus, the commissural nucleus of the brain stem, and the skin (Stengaard-Pedersen and Larsson, 1981; Nagahama et al, 1998; Hadley and Haskell-Luevano, 1999). In the spinal cord, immunoreactivity for the POMC-derived peptides ACTH and α -MSH has been detected in the dorsal horn and lamina X (Tsou et al, 1986).

Melanocortin Receptors and Ligands—Melanocortins exert their diverse biological effects by binding to a distinct family of receptors belonging to the G-protein coupled receptors, which display 7 transmembrane regions as a hallmark. Five melanocortin receptors have been identified (MC-1 to MC-5) corresponding to the products of 5 separate genes with a highly conserved amino acid identity (Hadley and Haskell-Luevano, 1999; Adan and

Gispén, 2000; Wikberg et al, 2000). Their activation leads to elevation of intracellular cyclic adenosine monophosphate through the activation of adenylate cyclase. While ACTH activates all 5 melanocortin receptors, α -MSH activates all receptors except the MC-2 receptor (Abdel-Malek, 2001). A unique property of the melanocortin system is the existence of endogenous antagonists of the melanocortin receptors (Lu et al, 1994). Indeed, hypothalamic neurons produce potent and selective antagonists of MC-3 and MC-4 receptors. These molecules, known as agouti or agouti-related peptide (AgRP), are expressed only in the arcuate nucleus of the hypothalamus by the same neurons that express neuropeptide Y (Shutter et al, 1997).

In order to study the specific role of each melanocortin receptor subtype in the regulation of various physiological functions (Table), several highly potent and selective agonist and antagonist analogues have been generated using substitution of residues in the endogenous ligands. Although several analogues have been reported in the literature, the description here will be limited to those that have been studied for their effects on sexual functions. Melanotan-II (MT-II), which emerged from early studies, has helped our understanding of the physiological role of MC-3 and MC-4 receptors in the control of penile erection. This compound is a cyclic peptide analogue of α -MSH that expresses agonistic activity at the MC-4 receptor, which is also an effective agonist on MC-1, MC-3, and MC-5 receptors (Wikberg et al, 2000). PT-141, which is the first metabolite of MT-II, is another synthetic peptide analogue with an agonist activity on melanocortin receptors, including the MC-3 and MC-4 receptors (Molinoff et al, 2003). Recently, a high-affinity tetrahydroisoquinoline (THIQ) agonist for the MC-4 receptor has been described (Sebhat et al, 2002). This ligand has a greater than 100-fold selectivity for the MC-4 receptor over the other melanocortin receptors. The group of synthetic melanocortin receptor antagonists is represented by SHU9119 and two HS compounds (Fan et al, 1997; Kask

et al, 1998; Schioth et al, 1999; Wikberg et al, 2000). SHU9119 antagonizes the MC-4 receptor at least threefold more potently than the MC-3 receptor. HS014 is a potent and selective antagonist of the MC-4 receptor. This ligand has approximately 20-fold higher affinity for the MC-4 receptor than the MC-3 receptor. Another antagonist selective for the MC-4 receptor is HS024, which is about 10-fold more potent for the MC-4 receptor compared to HS014.

The MC-3 and MC-4 receptors are abundantly expressed in the CNS, where they play a pivotal role in the regulation of feeding behavior and energy homeostasis. As shown in the Figure, these receptors are located in several nuclei involved in the control of erectile function. Expression of the MC-3 receptor is found mainly in the hypothalamus, thalamus, brainstem, and cortex (Roselli-Rehfuß et al, 1993; Lindblom et al, 1998), whereas the MC-4 receptor has a wider distribution and is found essentially in all regions of the brain, including the cortex, thalamus, hypothalamus, and brainstem (Mountjoy et al, 1994; Kishi et al, 2003). In the spinal cord, the MC-4 receptor was the only melanocortin receptor subtype for which mRNA was detectable. Mountjoy and Wild (1998) found that the MC-4 receptor mRNA was expressed throughout the spinal cord of the rat. Strong expression was observed in the intermediate zone of the thoracic spinal cord, particularly its lateral part, and in the ventral horn. A strong and dense signal was also observed over the outer part of the dorsal horn, and diffuse labeling was observed in the dorsal root ganglia. Over several lower segments of the spinal cord, a strong, dense signal was distributed as a continuous column in the gray matter on either side of the midline. Van der Kraan et al (1999) analyzed the expression of the MC-4 receptor in rat lumbar spinal cord (L₄–L₆) and found that levels of expression were greatest in area X and substantia gelatinosa; intermediate in laminae III, IV (medial aspect), V–VI, VII, and VIII; and low in the region of the ventrolateral motor neurons (lamina IX). MC-4 receptor mRNA *in situ* signal was observed throughout the gray matter, but a dense signal was also observed in lamina I–II.

Surprisingly, in male rats, the MC-4 receptor was also found in several peripheral tissues that have been implicated in the control of penile erection, including the penis itself, and the major pelvic ganglion (the equivalent in the rat of the pelvic plexus in humans, which is a major relay center for the autonomic pathways to the penis). More detailed mapping of the tissue distribution of MC-4 receptor using *in situ* hybridization was possible in human and rat penis, and MC-4 receptor mRNA was found at free nerve endings and mechanoreceptors in both the corpus cavernosum and glans of the penis (Van der Ploeg et al, 2002). This intriguing finding suggests that melanocortins may contribute to the control of erectile function

and male sexual behavior through peripheral action. The presence of MC-5 receptors in rat reproductive exocrine glands also supports the idea of functional coherence between the peripheral and central effects of the melanocortin peptides in the regulation of sexual behavior (van der Kraan et al, 1998).

Melanocortin System and Erectile Function

Melanocortins and Erection—The ability of ACTH and α -MSH to cause sexual excitation has been established in different species, including rats, rabbits, cats, dogs, and monkeys. In a number of reports, cerebroventricular injection of ACTH or α -MSH in a low range (1–10 μ g) has been shown to induce penile erection, stretching, and yawning (Bertolini and Gessa, 1981; Serra et al, 1987; Argiolas et al, 2000) in conscious animals. In rabbits, the sexual response beginning 15 to 60 minutes after administration of ACTH or α -MSH is characterized by recurrent episodes of penile erection accompanied by copulatory movements, ending with ejaculation (Bertolini and Gessa, 1981). In sexually experienced male rats, ACTH decreases the ejaculation latency and the number of mounts and intromissions prior to ejaculation during mating experiments. The effects of melanocortins on erection appear to be androgen-dependent because the erectile effect of ACTH was abolished by castration and restored by testosterone replacement (Bertolini and Gessa, 1981; Bertolini et al, 2001).

Erections induced by direct administration of ACTH and α -MSH in the lateral or third cerebral ventricles indicate the pivotal role of the brain in this response. Furthermore, intracerebral injection of very low doses of MT-II (Wessels et al, 2003), THIQ (Martin et al, 2002), and PT-141 (Molinoff et al, 2003) recapitulate the erectogenic effects of systemic administration of these agents. The role of the brain is also demonstrated by erections induced by both ACTH and α -MSH when administered directly into the hypothalamic periventricular region of the third ventricle, including the paraventricular nucleus of the hypothalamus, the dorsomedial nucleus, the ventromedial nucleus, and the anterior hypothalamic area. However, involvement of the paraventricular nucleus in mediating the proerectile effect of ACTH is unlikely, because lesion of this nucleus prevented yawning and penile erection induced by apomorphine (or oxytocin), but not by ACTH (Argiolas et al, 1987a). In contrast, hypophysectomy prevented ACTH- and apomorphine-induced penile erection in rats, but the accuracy of such surgery remains questionable (Serra et al, 1983, 1987).

Recently, identification of MC-4 receptors in the spinal cord has led to the investigation of the effects of intrathecal administration of melanocortins on erection. In anesthetized rats, no change in intracavernous pressure was found when α -MSH (3 μ g) was given intrathecally

(Mizusawa et al, 2002). In contrast with this report, Wessells and associates (2003) have reported that intrathecal administration of MT-II in conscious rats induced a dose-dependent increase in the occurrence of penile erections. Surprisingly, MT-II at the same doses was found to be more efficient in inducing erections when administered intrathecally than intracerebroventricularly. The reasons for the discrepancy between the two above studies remain unknown, but it may relate to the presence of anesthesia in the former or to a difference in the intrathecal site of injection. The differences between the melanocortin analogues used in each study may also account for these contrasting results. Indeed, in contrast to α -MSH, MT-II induces proerectile effects at very low doses (0.025 mg/kg, subcutaneously). We have recently confirmed the proerectile inducer effect of MT-II when delivered intrathecally in anesthetized rats (unpublished results).

As mentioned above, the presence of the melanocortin receptors in the free nerve endings and mechanoreceptors of erectile tissues is suggestive of an additive role of the melanocortin peptides in the control of penile erection via a peripheral action. However, Wessells (2003) found that intracavernous administration of MT-II did not result in any significant increase in penile intracavernosal pressure in anesthetized rats. In this study, intracavernous MT-II also had no effect on the erectile responses elicited by an electrical stimulation of the cavernous nerve, which led the authors to exclude any facilitatory effect of this agonist of the MC-4 receptor on the intrapenile mechanisms of erection. These findings are in agreement with *in vitro* studies in which MT-II failed to affect the electrical field stimulation-evoked relaxation of rabbit cavernosal strips (Vemulapalli et al, 2001). Similarly, the MC-4 receptor agonist THIQ did not affect baseline tension, nor did it relax isolated rat erectile tissue preparations (Van der Ploeg et al, 2002). The results of these studies would indicate that melanocortin receptors present in erectile tissue are not involved in the erectogenic effect of the melanocortins. However, given that MC-4 receptors appear to be expressed in the sensory fibers, it cannot be excluded that melanocortins may promote erection through an effect on the afferent nerve activity.

The available experimental results to date indicate that melanocortins promote erection primarily, if not solely, through a CNS action. Several aspects of this interaction between the melanocortin system and erectile function remain poorly explored, however. For example, the effect of melanocortins on penile erection in the presence or absence of sexual stimulation has not been extensively investigated. In this regard, we have recently observed that in anesthetized rats, intravenous administration of MT-II is 1) able to elicit erectile responses, and 2) enhance the erectile responses elicited by cavernous nerve stimulation (unpublished data). It is unclear whether these

two effects (ie, inducer/initiator and facilitator/conditioner) are due to the activation of the same mechanism or whether they involve the stimulation of different pathways. Whether the same melanocortin receptor subtype mediates these effects also remains unknown.

Receptors Involved in the Erectogenic Effects of Melanocortins—The abundant expression of MC-3 and MC-4 receptors in regions of the brain involved in the control of sexual behavior was the first indication that these two receptor subtypes had a functional role. Furthermore, the observation that inhibition of MT-II-induced increases in intracavernosal pressure by central neural administration of the nonselective antagonist SHU9119 provided further evidence for the role of these two receptor types in mediating the erectogenic effects of melanocortins (Wessells et al, 2003). Subsequently, a series of experiments have been carried out by several investigators in order to further elucidate the role of each receptor subtype in the control of erection. Using the selective MC-4 receptor antagonist HS014, it has been shown that grooming, stretching, and yawning induced by ACTH and α -MSH are mediated by the MC-4 receptor, whereas penile erection was not (Vergoni et al, 1998). Based on these results, it has been suggested that the MC-3 receptor may mediate the proerectile effects of melanocortins. However, data demonstrating the proerectile action of a selective MC-3 receptor agonist or the effects of selective blockade of MC-3 receptor on the erectile response to melanocortins are missing (Dines et al, 2003).

In contrast, much more data exist to support a role for the CNS MC-4 receptor in the erectogenic effects of melanocortins. Indeed, selective activation of the MC-4 receptor using the THIQ compound facilitates erection in an anesthetized mouse cavernous nerve-stimulated model (Van der Ploeg et al, 2002). The ability of the MC-4 receptor-selective agonist THIQ to enhance erectile responses was impaired in MC-4 receptor-knockout mice, which indicates that the effect observed is MC-4 receptor-mediated. Furthermore, intravenous administration of THIQ increased the intracavernosal pressure recorded telemetrically (Martin et al, 2002). Using the *ex copula* model (in which a penile erection is evoked in a conscious rat by retraction of the penile sheath), THIQ compound was also found to increase the number of penile erections in a dose-dependent manner (Martin et al, 2002). Finally, cerebroventricular administration of an MC-4 receptor-preferring antagonist blocked the proerectile effects of THIQ.

The ability of SHU9119 administered intrathecally to block the proerectile effects of MT-II, administered by the same route, suggests that the spinal action of MT-II is specifically mediated by the melanocortin receptors and not by other proerectile receptors (Wessells, 2003). The MC-4 receptor is the most likely candidate because it is

the only melanocortin receptor subtype that has been located in the spinal cord. The use of more selective analogues, or knockout mice (or both) should shed further light on this issue.

The diminished copulatory behavior of MC-4 receptor-knockout mice is an additional argument in favor of a role for the MC-4 receptor subtype in the control of male sexual function. Indeed, Van der Ploeg and associates (2002) have paired male MC-4 receptor-null and wild-type mice with estrous females, and found that MC-4 receptor-null mice have an increased mounting and intromission latency compared with control littermate mice of the wild type. Moreover, MC-4 receptor-knockout mice exhibited a reduced ejaculatory efficiency in that none of the MC-4 receptor-null animals ejaculated during a 1-hour observation period, compared with 5 of 11 wild-type mice. However, given that the MC-4 receptor-knockout mice are obese, it is difficult to make a definitive conclusion regarding whether the reduced copulatory behavior described in these mice is due to the absence of the MC-4 receptor or to the presence of obesity, which is known to decrease sexual activity.

Mechanisms Mediating the Proerectile Effects of Melanocortins—The mechanisms involved in the regulation of penile erection by melanocortins are not well characterized. Some investigators have shown that melanocortins may recruit a different circuit from the one used by apomorphine. This is based on the observation that cerebroventricular injection of an oxytocin antagonist can prevent the erectile activity induced by subcutaneous apomorphine or central neural oxytocin, but not the erectile activity induced by ACTH (Argiolas et al, 1987b). Similarly, Mizusawa et al (2002) found that α -MSH-induced erectile responses were independent of oxytocinergic mechanisms, because an antagonist to the oxytocin receptor had no effect on the erectile responses to α -MSH. In contrast to these findings, Martin et al (2002) have shown that systemic administration of a selective oxytocin receptor antagonist, L-368899, significantly attenuated the proerectile effects of the MC-4 receptor agonist THIQ. Furthermore, intracerebroventricular administration of L-368899 completely blocked the penile erections induced by THIQ. These data suggest that the oxytocinergic system may play a pivotal role in the control of erectile function by the MC-4 receptor. Further studies are required to explore the discrepancy between the role of the oxytocinergic pathway in the erectile effects of THIQ vs ACTH and α -MSH.

Several studies have shown the involvement of various neurotransmitters and neuromodulators in the control of erectile function by melanocortins. Indeed, the penile erections induced by ACTH were prevented by pretreatment with the nitric oxide synthase inhibitor L-NAME (Poggioli et al, 1995). Therefore, it has been concluded

that activation of nitric oxide in the CNS may represent a common pathway in the control of erection by melanocortins, oxytocin, and dopamine. Calcium channels also seem to be involved in melanocortin-induced penile erection because intracerebroventricular administration of the N-type calcium channel blocker ω -conotoxin prevented the erectile effects of ACTH (Argiolas et al, 1990). Microinjection of ω -conotoxin in the paraventricular nucleus of the hypothalamus failed, however, to alter the erection induced by ACTH (Argiolas et al, 1990). There is also evidence implicating other mechanisms in the control of erection by melanocortins as evidenced by the fact that penile responses to ACTH are inhibited by potassium channel openers (Vergoni et al, 1995) and muscarinic receptor antagonists (Ferrari et al, 1963). Finally, melanocortins may stimulate several other distinct and yet undiscovered mechanisms in the CNS that lead to the occurrence of a penile erection.

Melanocortin Agonists for the Treatment of Erectile Dysfunction

There has been much interest of late in the utilization of selective melanocortin receptor agonists in the treatment of ED. There is active research for new and selective analogues of the melanocortin receptors. Two melanocortin receptor agonists, MT-II and PT-141 (the deaminated metabolite of MT-II) have undergone early clinical trial examination for this indication.

Clinical Results with MT-II—MT-II-induced erections were first reported as an adverse event in a preliminary clinical study designed to evaluate the effect of MT-II on skin pigmentation in healthy subjects (Dorr et al, 1996). As a result, the effects of MT-II on ED were then specifically evaluated by Wessells et al (1998, 2000). In a double-blind, placebo-controlled crossover study of 10 men with psychogenic ED (Wessells et al, 2000), MT-II and vehicle placebo (saline solution) were each administered twice by subcutaneous injection for a total of 4 injections without visual sexual stimulation. Placebo and MT-II were separated by at least 24 hours. Of the 10 men with psychogenic ED who received MT-II at doses ranging from 0.025 to 0.157 mg/kg, 8 had rigid erections (as measured by RigiScan; Timm Medical Technologies, Eden Prairie, Minn). The time to onset of erection ranged from 15 to 270 minutes, and the erections lasted an average of 144 minutes.

This proerectile effect of MT-II does not appear to be limited to cases of psychogenic ED. Indeed, it has been shown that a similar dosing of MT-II is effective in organic ED (Wessells et al, 2000). In this latter study of 10 patients with organic ED, 9 reported, subjectively, an erection following at least one of two injections of MT-II (again without visual sexual stimulation). The duration of erections after MT-II administration averaged 64.1

minutes, ranging from 2 minutes to 4 hours of intermittent erectile activity. In this study, subjects were instructed to rate their level of sexual desire during the 6 hours following drug administration. The erectogenic effects of MT-II were associated with a significant increase in sexual desire after 13 (68%) of 19 doses, compared to 4 (19%) of 21 placebo administrations.

Overall, these studies have clearly demonstrated that MT-II exerts a proerectile activity in men with ED of various origin. The enhanced sexual desire reported with MT-II warrants further investigation. Nausea, stretching, yawning, and decreased appetite were reported more frequently after injections of MT-II than placebo, and only one subject experienced severe effects. The adverse event profile of this agonist included nausea, stretching, yawning, and decreased appetite. Although generally well-tolerated, one patient complained of episodes of nausea associated with vomiting. The adverse event profile of MT-II does, however, raise questions about the general safety and potential clinical utility of this drug.

Clinical Results with PT-141—The effect of PT-141 on penile erection in humans has been recently reported by Molinoff et al (2003). PT-141 was administered intranasally at doses ranging from 4 to 20 mg to 24 healthy subjects in a randomized, double-blind, placebo-controlled study, and the efficacy of the drug was evaluated by employing a RigiScanR (Dacomed, Minneapolis, Minn). This study was also carried out without visual sexual stimulation. Pharmacokinetic assessments showed that concentration of PT-141 increased in a dose-dependent manner, reaching a maximum 30 minutes after the administration with a half-life of 2 hours. The pharmacodynamics, particularly time to onset of erectile activity (34 to 63 minutes after treatment), correlated well with the known pharmacokinetics of PT-141. Compared to placebo-treated subjects, PT-141 significantly increased erectile activity. Indeed, the duration of erections with rigidity greater than 60% base was approximately 140 minutes in the subjects treated with 20 mg of PT-141 compared to 22 minutes in the placebo-treated group.

The effect of PT-141 (20 mg) has also been reported in a placebo-controlled trial of 24 men with mild to moderate ED (Molinoff et al, 2003). This study employed visual sexual stimulation (erotic films). A threefold increase in erectile activity was observed in subjects given PT-141 as compared to placebo. The duration of erection and penile rigidity were also significantly increased after PT-141 administration. These preliminary results in a population of men with ED provide support for the potential of PT-141. It should be noted that to date, no serious side effects have been reported after PT-141 administration in either normal subjects or in patients with ED.

Concluding Remarks

The important role of the melanocortinergetic system in the regulation of erectile function is now well recognized. Melanocortins control penile erection through an action in the CNS, but the precise role of the spinal and supraspinal centers and pathways in this regulation are not fully understood. The presence of melanocortin receptors in the peripheral nerve terminals of the penis suggest that they may also act through the peripheral nervous system. There are also controversial and conflicting observations regarding the melanocortin receptor subtypes involved in sexual function. Some data suggest that the MC-3 receptor mediates the proerectile effects of melanocortins, whereas other data suggest the predominant involvement of the MC-4 receptor. A synergism between the MC-3 and MC-4 receptors in the control of erectile function may also exist because dual stimulation of each subtype appears to produce greater than additive activation of erectile activity (Avis, 2000). The underlying mechanisms that mediate the proerectile effects of the melanocortins are also poorly understood. Detailed knowledge of the mechanism of action and the systems involved will be of importance for the discovery of new and more efficient drugs for the treatment of ED. For example, unraveling the receptor subtype that mediates the effects of the melanocortins on erection could potentially lead to a drug targeted specifically to this receptor.

From a clinical perspective it is also important to understand whether the melanocortins facilitate or induce erections (or both). This potential for inducer or initiator action is an important and perhaps unique pharmacological property that might be exploited either in monotherapy or as an adjunct to current phosphodiesterase type 5 inhibitor therapy. The effect of melanocortins on sexual desire is also questionable and deserves further investigation.

Early clinical trials have demonstrated both general efficacy and safety of MT-II and PT-141 in treating men with ED. It appears that PT-141 may be the preferable of the two agents based on initial safety experiences. Currently, many highly selective melanocortin receptor agonist molecules may be in preclinical development. Many of these molecules are, in fact, derived from obesity research. An important aspect of this research centers on the issue of the low bioavailability of peptidergic analogues after oral administration and the search for non-peptidergic ligands for melanocortin receptors that might eventually be administered orally. This class of drug offers the first new and potentially broadly effective and well-tolerated centrally acting drugs for the treatment of ED.

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References

- Abdel-Malek ZA. Melanocortin receptors: their functions and regulation by physiological agonists and antagonists. *Cell Mol Life Sci.* 2001; 58:434–441.
- Adan RA, Gispen WH. Melanocortins and the brain: from effects via receptors to drug targets. *Eur J Pharmacol.* 2000;405:13–24.
- Andersson KE. Pharmacology of penile erection. *Pharmacol Rev.* 2001; 53:417–450.
- Argiolas A, Melis MR, Mauri A, Gessa GL. Paraventricular nucleus lesion prevents yawning and penile erection induced by apomorphine and oxytocin but not by ACTH in rats. *Brain Res.* 1987a;421:349–352.
- Argiolas A, Melis MR, Murgia S, Schiöth HB. 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.* 2000;51:425–431.
- Argiolas A, Melis MR, Stancampiano R, Gessa GL. Role of calcium in the expression of ACTH-induced stretching, yawning and penile erection. *Brain Res Bull.* 1990;24:853–856.
- Argiolas A, Melis MR, Vargiu L, Gessa GL. d(CH₂)₅Tyr(Me)-[Orn⁸]vasotocin, a potent oxytocin antagonist, antagonizes penile erection and yawning induced by oxytocin and apomorphine, but not by ACTH-(1–24). *Eur J Pharmacol.* 1987b;134:221–224.
- Avis NE. Sexual function and aging in men and women: community and population-based studies. *J Genet Specif Med.* 2000;3:37–41.
- Bertolini A, Gessa GL. Behavioral effects of ACTH and MSH peptides. *J Endocrinol Invest.* 1981;4:241–251.
- Bertolini A, Gessa GL, Ferrari C. Penile erection and ejaculation: a central effect of ACTH-like peptides in mammals. In: Sandler M, Gessa GL, eds. *Sexual Behavior: Pharmacology and Biochemistry.* New York: Raven Press; 2001:247–257.
- Dines KC, Gahman TC, Girten BE, et al, inventors. Melanocortin receptor-3 ligands to treat sexual dysfunction. US patent 6 534 50. March 2003.
- Dorr RT, Lines R, Levine N, Brooks C, Xiang L, Hrubby VJ, Hadley ME. Evaluation of melanotan-II, a superpotent cyclic melanotropic peptide in a pilot phase-I clinical study. *Life Sci.* 1996;58:1777–1784.
- Fan W, Boston BA, Kesterson RA, Hrubby VJ, Cone RD. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature.* 1997;385:165–168.
- Ferrari W, Gessa GL, Vargiu L. Behavioural effects induced by intracranially injected ACTH and MSH. *Ann N Y Acad Sci.* 1963;104: 330–345.
- Giuliano F. Phosphodiesterase type 5 inhibition in erectile dysfunction: an overview. *Eur Heart J.* 2002;4:H7–H12.
- Giuliano F, Allard J. Apomorphine SL (Uprima): preclinical and clinical experiences learned from the first central nervous system-acting ED drug. *Int J Impot Res.* 2002;14(suppl 1):S53–S56.
- Giuliano F, Bernabé J, Brown K, Droupy S, Benoit G, Rampin O. Erectile response to hypothalamic stimulation in rats: role of peripheral nerves. *Am J Physiol.* 1997;273:R1990–R1997.
- Giuliano F, Rampin O. Central neural regulation of penile erection. *Neurosci Biobehav Rev.* 2000;24:517–533.
- Giuliano FA, Rampin O, Benoit G, Jardin A. Neural control of penile erection. *Urol Clin North Am.* 1995;22:747–766.
- Hadley ME, Haskell-Luevano C. The proopiomelanocortin system. *Ann N Y Acad Sci.* 1999;885:1–21.
- Hedlund H, Andersson KE. Contraction and relaxation induced by some prostanoids in isolated human penile erectile tissue and cavernous artery. *J Urol.* 1985;134:1245–1250.
- Kask A, Mutulis F, Muceniece R, Pahkla R, Mutule I, Wikberg JE, Rago L, Schiöth HB. Discovery of a novel superpotent and selective melanocortin-4 receptor antagonist (HS024): evaluation in vitro and in vivo. *Endocrinology.* 1998;139:5006–5014.
- Kishi T, Aschkenasi CJ, Lee CE, Mountjoy KG, Saper CB, Elmquist JK. Expression of melanocortin 4 receptor mRNA in the central nervous system of the rat. *J Comp Neurol.* 2003;457:213–235.
- Lindblom J, Schiöth HB, Larsson A, Wikberg JE, Bergstrom L. Autoradiographic discrimination of melanocortin receptors indicates that the MC3 subtype dominates in the medial rat brain. *Brain Res.* 1998; 810:161–171.
- Lu D, Willard D, Patel IR, et al. Agouti protein is an antagonist of the melanocyte-stimulating-hormone receptor. *Nature.* 1994;371:799–802.
- Martin WJ, McGowan E, Cashen DE, et al. Activation of melanocortin MC(4) receptors increases erectile activity in rats ex copula. *Eur J Pharmacol.* 2002;454:71–79.
- McKenna K. The brain is the master organ in sexual function: central nervous system control of male and female sexual function. *Int J Impot Res.* 1999;(suppl 1):S48–S55.
- Mizusawa H, Hedlund P, Andersson KE. alpha-Melanocyte stimulating hormone and oxytocin induced penile erections, and intracavernous pressure increases in the rat. *J Urol.* 2002;167:757–760.
- Molinoff PB, Shadiack AM, Earle D, Diamond LE, Quon CY. PT-141: a melanocortin agonist for the treatment of sexual dysfunction. *Ann N Y Acad Sci.* 2003;994:96–102.
- Moreland RB, Nakane M, Hsieh G, Brioni JD. Perspectives for pharmacotherapy of male erectile dysfunction. *Curr Opin CPNS Investig Drugs.* 2000;2:283–302.
- Mountjoy KG, Mortrud MT, Low MJ, Simerly RB, Cone RD. Localization of the melanocortin-4 receptor (MC4-R) in neuroendocrine and autonomic control circuits in the brain. *Mol Endocrinol.* 1994;8:1298–1308.
- Mountjoy KG, Wild JM. Melanocortin-4 receptor mRNA expression in the developing autonomic and central nervous systems. *Brain Res Dev Brain Res.* 1998;107:309–314.
- Nagahama M, Funasaka Y, Fernandez-Frez ML, Ohashi A, Chakraborty AK, Ueda M, Ichihashi M. Immunoreactivity of alpha-melanocyte-stimulating hormone, adrenocorticotrophic hormone and beta-endorphin in cutaneous malignant melanoma and benign melanocytic naevi. *Br J Dermatol.* 1998;138:981–985.
- Poggioli R, Benelli A, Arletti R, Cavazzuti E, Bertolini A. Nitric oxide is involved in the ACTH-induced behavioral syndrome. *Peptides.* 1995;16:1263–1268.
- Rampin O, Bernabé J, Giuliano F. Spinal control of penile erection. *World J Urol.* 1997;15:2–13.
- Roselli-Rehffuss L, Mountjoy KG, Robbins LS, et al. Identification of a receptor for gamma melanotropin and other proopiomelanocortin peptides in the hypothalamus and limbic system. *Proc Natl Acad Sci U S A.* 1993;90:8856–8860.
- Saenz de Tejada I. Molecular mechanisms for the regulation of penile smooth muscle contractility. *Int J Impot Res.* 2002;14(suppl 1):S6–S10.
- Schiöth HB, Muceniece R, Mutulis F, Bouifrouri AA, Mutule I, Wikberg JE. Further pharmacological characterization of the selective melanocortin 4 receptor antagonist HS014: comparison with SHU9119. *Neuropeptides.* 1999;33:191–196.
- Schmidt MH, Schmidt HS. The ischiocavernosus and bulbospongiosus muscles in mammalian penile rigidity. *Sleep.* 1993;16:171–183.
- Sebhat IK, Martin WJ, Ye Z, et al. Design and pharmacology of N-[(3R)-1,2,3,4-tetrahydroisoquinolinium-3-ylcarbonyl]-(1R)-1-(4-chlorobenzyl)-2-[4-cyclohexyl-4-(1H-1,2,4-triazol-1-ylmethyl)piperidin-1-yl]-2-

- oxoethylamine (1), a potent, selective, melanocortin subtype-4 receptor agonist. *J Med Chem.* 2002;45:4589–4593.
- Serra G, Collu M, Loddo S, Celasco G, Gessa GL. Hypophysectomy prevents yawning and penile erection but not hypomotility induced by apomorphine. *Pharmacol Biochem Behav.* 1983;19:917–919.
- Serra G, Fratta W, Collu M, Gessa GL. Hypophysectomy prevents ACTH-induced yawning and penile erection in rats. *Pharmacol Biochem Behav.* 1987;26:277–279.
- Shutter JR, Graham M, Kinsey AC, Scully S, Luthy R, Stark KL. Hypothalamic expression of ART, a novel gene related to agouti, is up-regulated in obese and diabetic mutant mice. *Genes Dev.* 1997;11:593–602.
- Stackl W, Hasun R, Marberger M. Intracavernous injection of prostaglandin E1 in impotent men. *J Urol.* 1988;140:66–68.
- Stengaard-Pedersen K, Larsson LI. Comparative immunocytochemical localization of putative opioid ligands in the central nervous system. *Histochemistry.* 1981;73:89–114.
- Tsou K, Khachaturian H, Akil H, Watson SJ. Immunocytochemical localization of pro-opiomelanocortin-derived peptides in the adult rat spinal cord. *Brain Res.* 1986;378:28–35.
- van der Kraan M, Adan RA, Entwistle ML, Gispen WH, Burbach JP, Tatro JB. Expression of melanocortin-5 receptor in secretory epithelia supports a functional role in exocrine and endocrine glands. *Endocrinology.* 1998;139:2348–2355.
- van der Kraan M, Tatro JB, Entwistle ML, Brakkee JH, Burbach JP, Adan RA, Gispen WH. Expression of melanocortin receptors and pro-opiomelanocortin in the rat spinal cord in relation to neurotrophic effects of melanocortins. *Brain Res Mol Brain Res.* 1999;63:276–286.
- Van der Ploeg LH, Martin WJ, Howard AD, et al. A role for the melanocortin 4 receptor in sexual function. *Proc Natl Acad Sci U S A.* 2002;99:11381–11386.
- Vemulapalli R, Kurowski S, Salisbury B, Parker E, Davis H. Activation of central melanocortin receptors by MT-II increases cavernosal pressure in rabbits by the neuronal release of NO. *Br J Pharmacol.* 2001;134:1705–1710.
- Vergoni AV, Bertolini A, Mutulis F, Wikberg JE, Schioth HB. Differential influence of a selective melanocortin MC4 receptor antagonist (HS014) on melanocortin-induced behavioral effects in rats. *Eur J Pharmacol.* 1998;362:95–101.
- Vergoni AV, Sandrini M, Filafarro M, Bertolini A. Opening of brain potassium-channels inhibits the ACTH-induced behavioral syndrome in the male rat. *Neurosci Lett.* 1995;188:29–32.
- Voisey J, Carroll L, van Daal A. Melanocortins and their receptors and antagonists. *Curr Drug Targets.* 2003;4:586–597.
- Wessells H, Fuciarelli K, Hansen J, Hadley ME, Hruby VJ, Dorr R, Levine N. Synthetic melanotropic peptide initiates erections in men with psychogenic erectile dysfunction: double-blind, placebo controlled crossover study. *J Urol.* 1998;160:389–393.
- Wessells H, Gralnek D, Dorr R, Hruby VJ, Hadley ME, Levine N. Effect of an alpha-melanocyte stimulating hormone analog on penile erection and sexual desire in men with organic erectile dysfunction. *Urology.* 2000;56:641–646.
- Wessells H, Hruby VJ, Hackett J, Han G, Balse-Srinivasan P, Vanderah TW. Ac-Nle-c[Asp-His-DPhe-Arg-Trp-Lys]-NH₂ induces penile erection via brain and spinal melanocortin receptors. *Neuroscience.* 2003;118:755–762.
- Wikberg JE, Muceniece R, Mandrika I, Prusis P, Lindblom J, Post C, Skottner A. New aspects on the melanocortins and their receptors. *Pharmacol Res.* 2000;42:393–420.