Bremelanotide: An Overview of Preclinical CNS Effects on Female Sexual Function

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DOI: 10.1111/j.1743-6109.2007.00610.x

ABSTRACT

Introduction. Bremelanotide is an analogue of the naturally occurring peptide α-melanocyte-stimulating hormone (α-MSH). It stimulates erection in men and male rats, and is currently in clinical trials for the treatment of erectile dysfunction.

Aim. To review the effects of bremelanotide, an analogue of the naturally occurring peptide α-MSH, on the preclinical indices of sexual desire in female rats, and where in the brain these actions may occur.

Main Outcome Measures. Appetitive sexual behaviors, such as solicitations, hops and darts, and pacing, were assessed along with consummatory behaviors such as lordosis. The involvement of brain regions was assessed following direct administration to the region, by the stimulation of molecular markers of neural activation, and using microdialysis to examine extracellular fluid for different neurotransmitters.

Methods. Using a model that allows ovariectomized, hormone-primed female rats to control the timing of sexual encounters with males, we tested the ability of bremelanotide to increase appetitive (proceptive) and/or consummatory sexual behaviors.

Results. Bremelanotide dramatically and selectively increased measures of solicitation in female rats, without altering pacing or lordosis, following both peripheral (subcutaneous) administration or infusions directly into the lateral ventricles or medial preoptic area (mPOA), but not the ventromedial hypothalamus. The mPOA is critical for the display of appetitive sexual behaviors in females and males of a variety of species. Peripheral administration of bremelanotide activates the mPOA and other hypothalamic and limbic regions of the brain involved in sexual behavior, and may work by activating dopamine terminals in the mPOA.

Conclusions. To the extent that solicitations indicate the desire of female rats to engage in sexual activity, bremelanotide appears to possess the behavioral, pharmacological, and neuroanatomical specificity required of a drug in the treatment of hypoactive sexual desire disorders. **Pfaus J, Giuliano F, and Gelez H. Bremelanotide: An overview of preclinical CNS effects on female sexual function. J Sex Med 2007;4(suppl 4):269–279.**

Key Words. Female Sexual Response; Preclinical Models; Melanocortins; Female Desire; Rat

Introduction: Animal Models of Sexual Behavior

Research on animal sexual behavior generally concerns the investigation of basic behavioral and physiological mechanisms, including neuroendocrine, neuroanatomical, neurochemical, and molecular functions. However, such research is also conducted to provide preclinical data on the impact of pharmacological agents, instrumentation, new devices, and/or surgical procedures, prior to clinical trials, or to examine sexual "side effects" of different treatments. In those latter cases, the animal models used must allow clinicians to extrapolate the preclinical results to human clinical populations, and in particular to determine the likelihood that a treatment will be successful or will warrant subsequent human trials [1,2].

In all species, sexual behavior is directed by a complex interplay between steroid hormone actions in the brain that give rise to sexual arousability, and experience with sexual reward or pleasure that gives rise to expectations of competent

sexual activity, including sexual arousal, desire, and elements of copulatory performance [3–5]. Sexual experience allows animals to form instrumental and Pavlovian associations that predict sexual outcomes and thereby direct the strength of sexual responding. Although the study of animal sexual behavior by neuroendocrinologists has traditionally been concerned with mechanisms of copulatory responding, more recent use of conditioning and preference paradigms, and a focus on environmental circumstances and experience, has revealed behaviors that are analogous or homologous to human sexual desire. All animals must be able to respond to hormonal and neurochemical changes that signal sexual arousal and desire, and be able to interact with external sexual incentives. Animals need to be able to identify external stimuli that predict where potential sex partners can be found, and subsequently seek them out, solicit, court, or otherwise work to obtain sex partners, distinguish cues and behavioral patterns of potential partners from those that are not interested or receptive, and pursue desired sex partners once sexual contact has been made. Sexual responding becomes habitual or automated with practice, and the processes that underlie this effect of experience make animals less affected by situations or treatments that disrupt sexual responding in sexually naive animals (e.g., [6]).

Similarly, neural and hormonal mechanisms exist that allow the stimulation received during sexual contact to be perceived as pleasurable or rewarding. Such reward alters subsequent behavior by facilitating the formation of place or partner preferences for salient stimuli associated with sexual reward (reviewed in Pfaus et al. [5]). Many of these aspects of sexual responding go beyond the traditional focus on copulation, penile reflexes, or lordosis in females. Indeed, although some appetitive and precopulatory sexual responses that animals make prior to copulation are not specific to sexual behavior (e.g., bar-pressing), they can be considered "sexual" if they are conditioned using sexual reward as the positive reinforcer (e.g., [7]).

Copulatory Behavior of the Female Rat

Although females and males of most mammalian species engage in mutual and complementary patterns of sexual activity, it is the females that have the final say in allowing successful sexual interaction. Females in the wild control virtually all aspects of sexual interaction, including the initiation and temporal patterning of copulation. This occurs by a complex interaction of appetitive behaviors that attract and solicit sex from males, pacing behaviors used to control the rate of copulation and sexual stimulation, receptive behaviors such as lordosis that allow penile intromissions to occur, and defensive behaviors used either to pace the copulatory contact if females cannot otherwise do so, or to terminate the contact. These behaviors serve to optimize the rate and strength of sexual stimulation received by females, which in turn initiates neuroendocrine reflexes associated with fertility and pregnancy [5,8,9].

Models of Sexual Desire

Like humans, animals manifest sexual excitement and desire behaviorally (Figure 1). They will increase their motor output in anticipation of copulation and work for the opportunity to copulate or to obtain primary or secondary (conditioned) sexual rewards associated with copulation. Animals will also choose between two or more sexual incentives based on the strength of the incentive cues and the animal's own internal drive state. What characterizes many of these behaviors is that they occur before copulation: courtship, operant responses, conditioned locomotion in anticipation of sex, time spent near a particular sexual incentive, or choices made between two or more incentives, can all be considered analogies of anticipatory sexual desire. The strength of the behavior can be observed as increases or decreases, or can be tested by increasing the criterion level of responding that animals must attain before they are given access to rewards. Simply put, animals with more "desire" will display more robust behavior than animals with less desire. Desire can also be inferred from certain copulatory behaviors, such as solicitation and pacing, in which female rats control the initiation and rate of the stimulation they receive (Figure 2). A growing body of evidence indicates that these aspects of sexual behavior are altered in a relatively selective fashion by hormones and known neurochemical circuits in the brain.

Steroid Modulation of Sexual Desire

The role of steroid hormones in sexual desire has been established by studies showing that castration in rats (removal of the testis in males and ovaries in females) results in animals that no longer seek out or work for sexual contact. In females, ovariectomy results in a complete cessation of both appetitive and consummatory sexual behaviors. Sexual

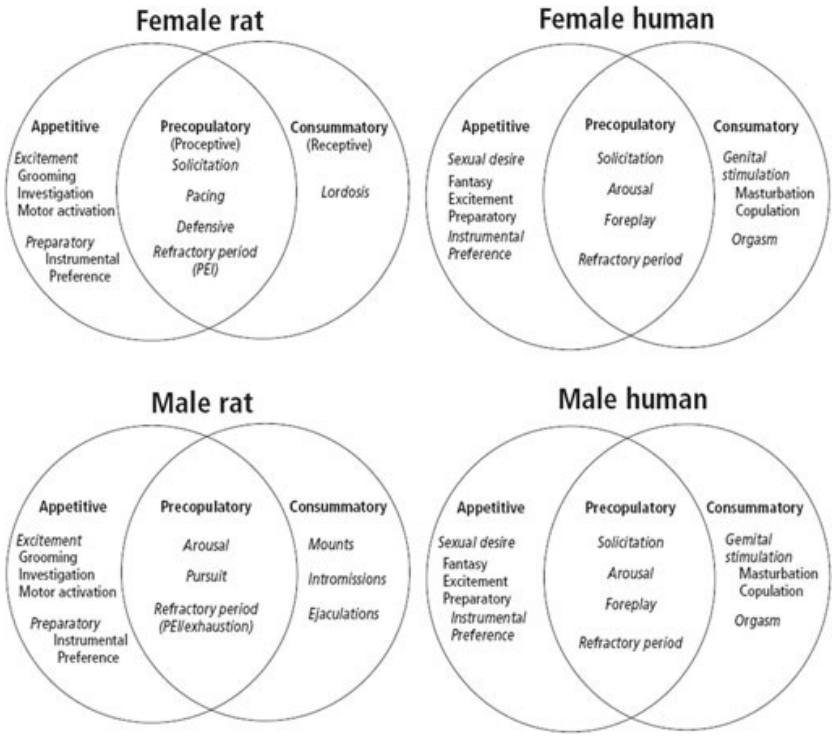


Figure 1 Incentive sequences for human and rat sexual behavior (modified from Pfaus, [10,11). The behavioral stream moves from left to right, through appetitive, precopulatory, and consummatory phases of behavior. This conforms to the movement of animals from distal to proximal to interactive with respect to the sexual incentive. Three types of appetitive responding reflect relative degrees of learning and necessity. "Preparatory" behaviors are learned responses that animals must make in order to acquire the incentive (e.g., operant behaviors, pursuit, etc.). "Anticipatory" behaviors are learned responses that occur in anticipation of an incentive, but are not necessary to obtain it (e.g., conditioned psychomotor stimulation that characterizes behavioral excitement). Unlearned appetitive responses also exist that are instinctual (e.g., unconditioned anogenital investigation). These aspects of behavior also occur once copulatory contact has been made, especially if copulation occurs in bouts (as it does in rats).

behavior is restored by sequential treatment with estradiol and progesterone [9] in a pattern that mimics the cyclic ovarian output of these steroids. Gonadally intact female rats cycle in 4- to 5-day intervals, defined by vaginal cell morphology as Diestrus, Metestrus, Proestrus, and Estrus. Sexual activity in wild rats begins in the early evening of Proestrus, and continues through the early morning of Estrus. This corresponds to the period a few hours after the progesterone surge that comes immediately after ovulation [12]. Estradiol binds in high density to the hypothalamus, regions of the limbic system and cortex, midbrain and brainstem, and initiates a cascade of protein synthetic changes that alters chemical transmission in the brain [9,13]. This is done through a complex series of changes in neurotransmitter synthesis, release, and binding, which sculpts a "sexual" brain, one that focuses attention on the search for, and interaction with, sexual incentives in the external world. A necessary step involves the synthesis of progestin receptors that bind progesterone, along with an increase in the actions of neurotransmitters like dopamine, noradrenaline, oxytocin, melanocortins, and gonadotrophin-releasing hormone that have global actions on sexual behavior and transmitters like glutamate and γ-aminobutyric acid (GABA) that have local actions in discrete hypothalamic regions to disinhibit or inhibit sexual behavior [13]. These neurochemical systems are thus thought of as intermediaries of estrogen actions that link sexual responding to the neuroendocrine and reproductive needs of the female.

Hypothalamic-Limbic Integration

Among the sites of estrogen and progesterone binding, two hypothalamic regions have emerged in the past 40 years as vitally important for the regulation of appetitive and consummatory sexual behaviors in female mammals, the ventromedial hypothalamus (VMH) and the medial preoptic area (mPOA). Estrogen implants in the VMH of

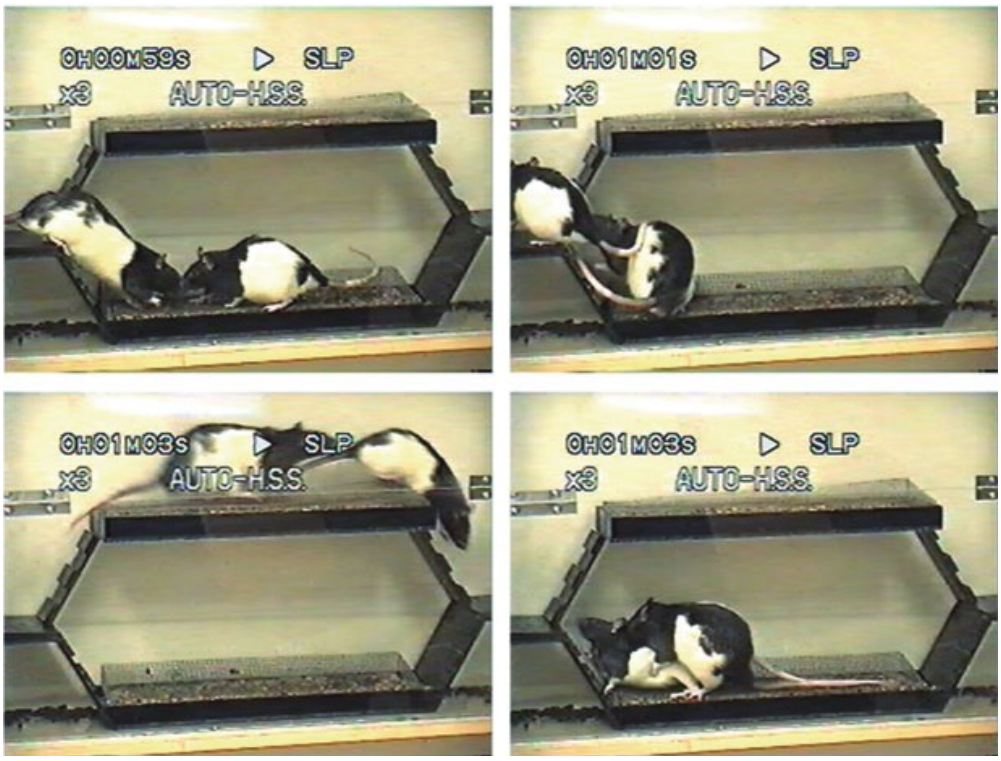


Figure 2 Bi-level chambers used to study appetitive and consummatory measures of copulation in rats. Depicted is a female soliciting a male (top left), then allowing him to investigate her anogenital region (top right), followed by a runaway to the top level, during which the male pursues her (bottom left), leading to lordosis, which allows the male to mount and gain vaginal penetration (bottom right). The entire bout of copulation took 4 seconds. Vaginal penetration is followed by an abrupt dismount, after which the male grooms his penis and anogenital regions. The male ejaculates after several copulatory bouts with intromission.

ovariectomized rats facilitate lordosis, and lesions of the VMH inhibit the display of lordosis in females treated with estradiol and progesterone [9]. The VMH receives noradrenergic input from the rostral brainstem, which activates lordosis relevant neurons there. Estradiol also potentiates GABAergic input to the VMH, which serves to inhibit glutamatergic interneurons that are inhibitory for both appetitive and consummatory sexual behaviors in females [14], and thus to disinhibit female sexual behavior. Conversely, the vaginocervical stimulation received by females from multiple penile intromissions during sexual interaction with males activates those glutamate interneurons selectively, and helps to bring about a state of sexual inhibition known as "estrus termination" (M. Georgescu, et al., unpublished data) [15]. In contrast, lesions of the mPOA inhibit virtually all appetitive sexual behaviors, such as solicitations and hops and darts, and disrupt the pacing of copulation in female rats [16], but either enhance or do not alter lordosis [17]. The VMH and mPOA are interconnected structures, and appear to regulate the temporal patterning of solicitations, pacing, and lordosis. The mPOA receives inputs from several important brain regions, such as the main olfactory (piriform) cortex, and sends outputs to the ventral tegmental area (VTA) in the midbrain, and the nucleus accumbens. The VTA gives rise to mesolimbic dopamine neurons that project to the nucleus accumbens. The mesolimbic (A10) dopamine system is vitally important in the transfer from motivation to action, and in particular in attention to incentive stimuli. Interestingly, in males, the pattern of dopamine release in the nucleus accumbens during copulatory behavior is identical to the pattern in the mPOA, although mPOA dopaminergic terminals come from cell bodies in the zona incerta, a subthalamic nucleus caudal to the hypothalamus that receives diffuse input from motor cortex and cerebellum. This dopamine (A14) system is not part of the mesolimbic system, although its modulation of output from the mPOA to the nucleus accumbens suggests that it may help to shape the particular responses of animals toward particular incentives. The integra-

tion of hypothalamic and limbic actions creates a system that, when modulated by steroid hormones, primes the animal's attention toward sexual stimuli and sexual interaction.

Interaction of Melanocortins with the Hypothalamic-Limbic Sexual System

The melanocortin family of neuropeptides is derived from the polypeptide precursor proopiomelanocortin (POMC). Prohormone-converting enzymes cleave POMC into several bioactive peptides including α-, β-, and γ-melanocyte-stimulating hormone (MSH), adrenocorticotropic hormone, and the opioid β-endorphin. The first two of these peptides interact with specific MC receptors. These peptides have pronounced effects on the sexual behavior of rats [18–20]. The role of MC receptors in the regulation of penile erection, has received increasing attention [21–23]. In female rats, α-MSH has been shown to facilitate or inhibit lordosis depending on the hormonal status of the animals [24–31]. Melanocortinergic neurons are found largely in the arcuate nucleus of the hypothalamus, and project widely throughout the hypothalamus and limbic system. Estradiol increases α-MSH levels in the hypothalamus [29,30], suggesting that α-MSH release may be one of several intermediaries of estrogen action. MSH binds to central and peripheral melanocortinergic receptors (MCRs), including MCR3 and MCR4 in the hypothalamus and limbic system. This prompted the study of the central actions of bremelanotide (formerly PT-141), a cyclic peptide analogue of α-MSH and the deaminated metabolite of melanotan-II (MT-II) [32] on the sexual behavior of female rats. Both bremelanotide and MT-II have a high affinity for MC1, MC3, MC4, and MC5 receptors.

Subcutaneous injections of bremelanotide produce a dramatic increase in solicitations and hops and darts in ovariectomized rats primed with estradiol and progesterone, or estradiol alone, without a corresponding decrease in pacing, or any effect on lordosis (Figures 3 and 4) [33]. Similar effects were reported with MT-II [34]. Females treated with the highest dose of bremelanotide also attempted to mount the males, a behavior that is typically observed in primed females when they are paired with castrated or sexually sluggish males, and is considered a measure of appetitive or proceptive sexual behavior [35]. Administration of bremelanotide produced no overall effects on general locomotion, indicating that the increase in solicitations was behaviorally specific, and not secondary to overall systemic stimulation. The effect of bremelanotide was consistent in both bi-level chambers and unilevel pacing chambers bisected by a divider with openings that only the female (and not the male) can cross, again indicating that

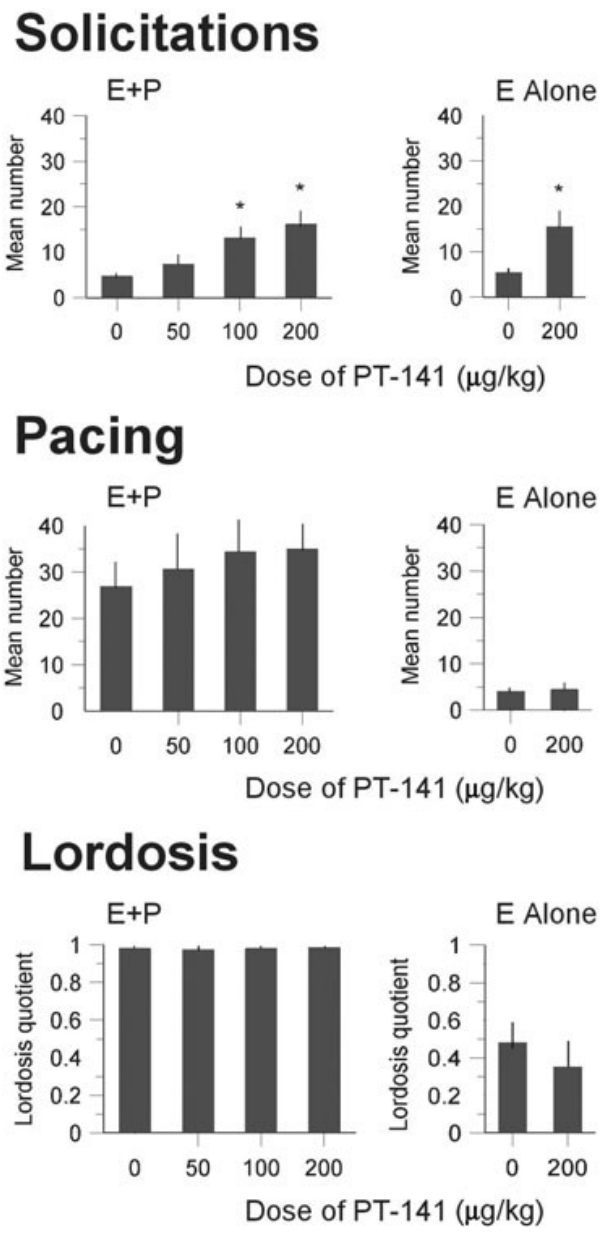


Figure 3 Dose–response effects of bremelanotide on female rat sexual behavior in bi-level chambers. Top: Effects on solicitations in females primed with estrogen and progesterone (E + P) or estrogen alone (E alone). Middle: Effects on pacing in females primed with E + P or E alone. Bottom: Effects on lordosis quotients in females primed with E + P or E alone. Data are means + standard error of the mean. $^*P < 0.05$ from control (analysis of variance followed by Tukey post hoc comparisons of individual means).

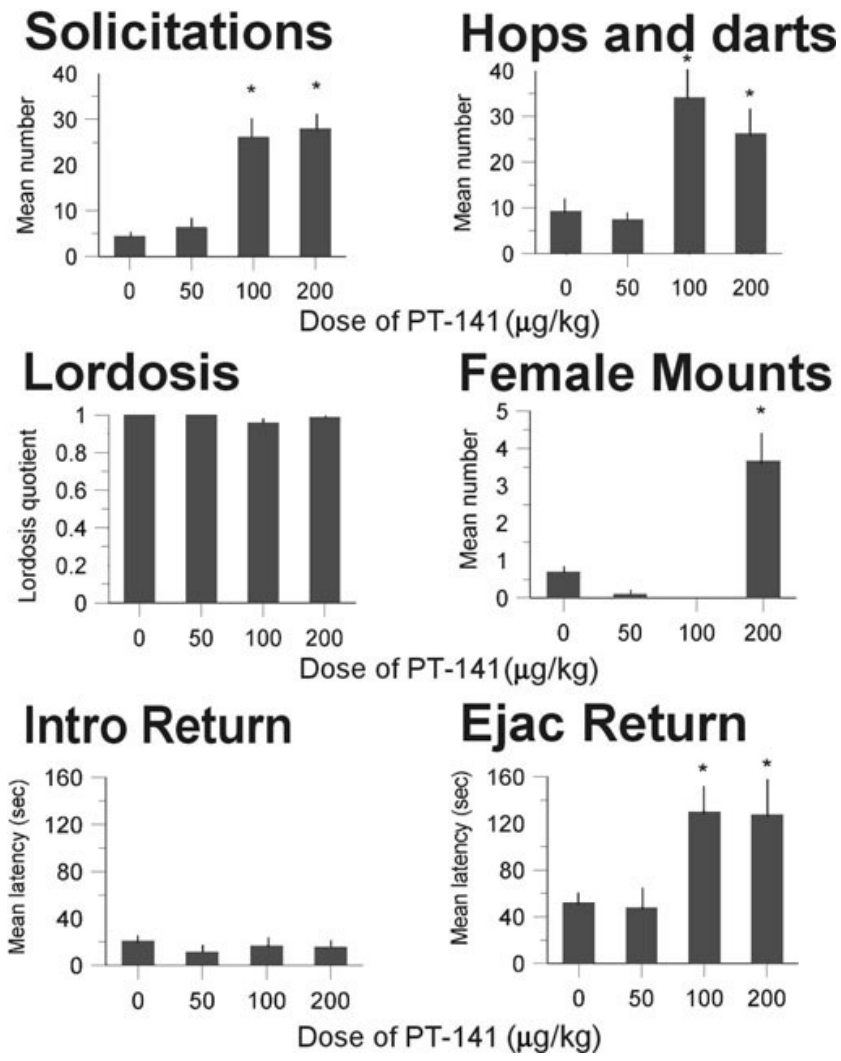


Figure 4 Dose–response effects of bremelanotide on the sexual behavior of estrogen and progesterone (E + P)-primed female rats in unilevel pacing chambers. Top left: Effects on solicitations. Top right: Effects on hops and darts. Middle left: Effects on lordosis quotients. Middle right: Effects on female mounting of the male. Bottom left: Effects on the intromission return latency, defined as the mean time it takes the female to return to the side of the male following an exit from the male's side. Bottom right: Effects on the ejaculation return latency, defined as the mean time it takes the female to return to the side of the male following an exit from the male's side. Data are means + standard error of the mean. $^*P < 0.05$ from control (analysis of variance followed by Tukey post hoc comparisons of individual means).

the effect was behavior-specific and not merely because of an overall increase in locomotion.

An identical effect of bremelanotide on behavior was found following infusions to the lateral ventricles or the mPOA, but not the VMH, both of which were reversed by infusions of an MC4 receptor antagonist (HS014) to the ventricles or mPOA (Figure 5). Similar effects were found with MT-II, suggesting (i) that peripheral administration of the peptides is acting in the brain to alter behavior, and (ii) that agonist activity at MC4 receptors in the mPOA is an important component of sexual solicitation. Similarly, subcutaneous injections of bremelanotide alone also induced the immediate-early gene product Fos in a variety of limbic and hypothalamic structures in female rats (Figure 6), including the nucleus accumbens, mPOA, paraventricular nucleus, basolateral amygdala, medial prefrontal cortex, and VTA, but not the VMH, suggesting that it may serve as a pharmacological "prime" to excite appetitive sexual activity in the presence of an appropriate sexual stimulus. Moreover, subcutaneous injections of bremelanotide increased dopamine release only in the mPOA, and not nucleus accumbens or VMH (Figure 7), suggesting that incertohypothalamic dopamine transmission may also be important for the increases in solicitation. Indeed, the increase in solicitations following peripheral injections of bremelanotide could be reversed with infusions of the dopamine D1 receptor antagonist flupenthixol into the mPOA, suggesting that melanocortin neurons make presynaptic contact with dopamine terminals to activate dopamine release selectively in this region of the brain. Interestingly, none of the melanocortins altered the expression of sexually conditioned place preference, in which females prefer places associated with paced vs. nonpaced copulation. Thus, the drug appears to stimu-

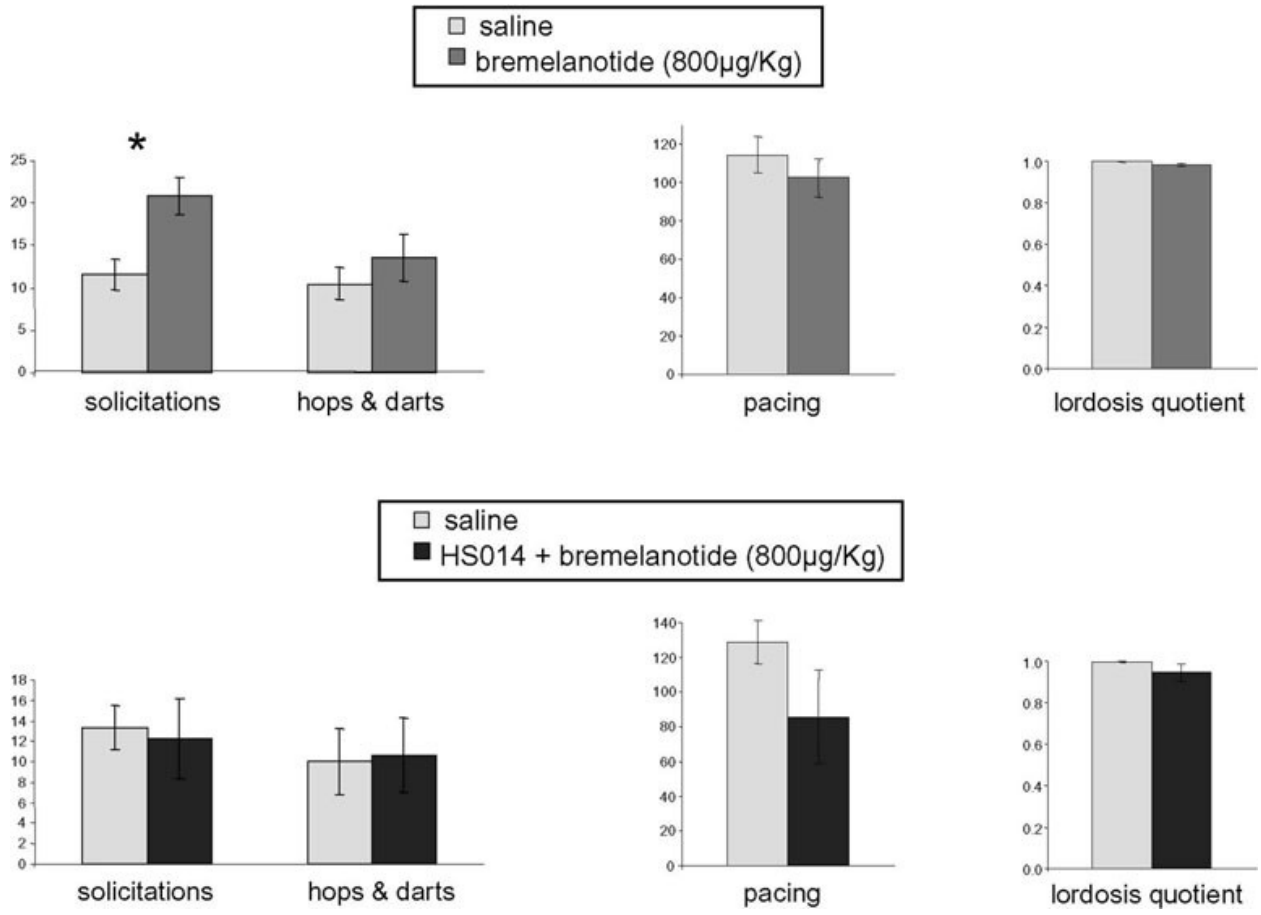
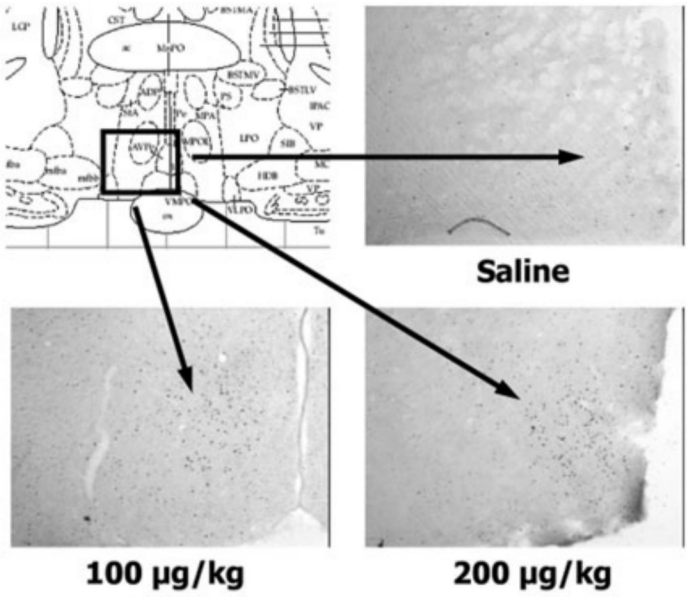


Figure 5 Effects of bilateral infusions of bremelanotide (800 µg) to the medial preoptic area of ovariectomized (OVX), estrogen-primed rats. Top: Effects on the mean number of solicitations, hops and darts, pacing, and the lordosis quotient (lordosis to mount ratio). Bottom: Effect on number of solicitations reversed with HS014, a selective MC4 receptor antagonist. Data are means + standard error of the mean. $^*P < 0.05$ from control (Student's *t*-test between the means).

late hormone-mediated neurochemical pathways involved in translating sexual desire into action, without making females less discriminative in their copulatory preferences.

Finally, although our data clearly indicate that peripherally administered bremelanotide (or MT-II) activates endogenous melanocortin receptors in the mPOA and elsewhere in the hypothalamic-limbic circuit that underlies female (and male) sexual responding, we cannot rule out the possibility that it also interacts with melanocortin receptors in the spinal cord and/or periphery. In male rats, infusions of MT-II to the spinal cord or lateral ventricles facilitated penile erection, as did intravenous injections, an effect that was blocked by the mixed MC3-MC4 receptor antagonist SHU9119 [37]. Although neither spinalization nor bilateral transection of pelvic nerves or dorsal penile nerves impaired the facilitation of erection following intravenous MT-II, this activity was abolished after acute removal of the lumbar paravertebral sympathetic chain [38]. This suggests that dual (and perhaps interactive and multiplicative) melanocortinergic mechanisms exist in spinal cord and brain to control penile erection. Indeed, yawning was also induced following infusions of MT-II to the lateral ventricles or intravenous injections, but not after infusions to the spinal cord [39]. This strongly suggests that the brain and spinal cord participate in translating melanocortin binding into behavior. Although direct injections to the corpus cavernosum did not facilitate erection in either study, MC4 receptor expression has been found in rat and human penis and penile pelvic ganglion (the major autonomic relay center to the penis), along with rat spinal cord, hypothalamus, limbic system and brainstem, pelvic ganglion (major autonomic relay center to the penis), but not in rat primary corpus smooth muscle cavernosum cells [22]. It is noteworthy that



Brain Region	Activity	Significance
mPOA	+++	Solicitation (Proceptivity)
VTA	+++	Incentive Salience Attention
NAc	+++/++ shell/core	Incentive Salience Attention
PFC	+++	Conditioned Arousal
BLA	+++	Reward-Related Learning
VMH	-	Lordosis

Figure 6 Effect of peripheral injections of saline or bremelanotide on Fos induction in the brain. Top: Dose–response effects on Fos induction in the medial preoptic area (mPOA). Top left is a drawing of a coronal section taken from the Swanson atlas [36] with the region of the mPOA depicted in the tissue sections within the black square. The section corresponds to Plate 20 of the atlas, with an anterior–posterior plane of section –0.46 from Bregma. Bottom: Qualitative stimulation of Fos in hypothalamic and limbic structures following treatment with the highest dose. ++ indicates at least a twofold increase in Fos positive cells induced by subcutaneous injection of the highest dose of bremelanotide relative to the saline control. +++ indicates at lease a fivefold increase. VTA = ventral tegmental area; NAc = nucleus accumbens; PFC = prefrontal cortex; BLA = basolateral amygdala; VMH = ventromedial hypothalamus.

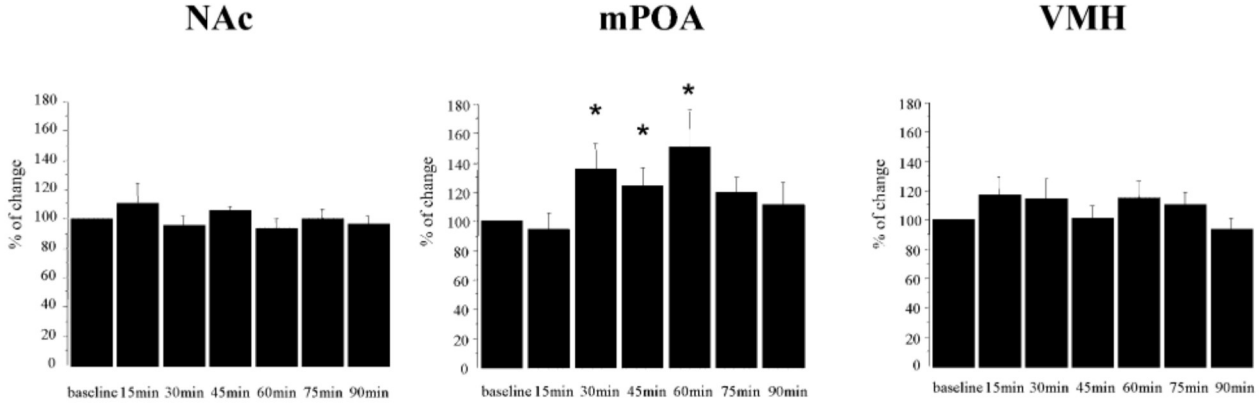


Figure 7 Dopamine release in the nucleus accumbens (NAc; left), medial preoptic area (mPOA; middle), and ventromedial hypothalamus (VMH; right) following peripheral administration of 200 µg/kg of bremelanotide. Data are mean percentages from baseline + standard error of the mean. $^*P < 0.05$ from baseline (analysis of variance followed by Tukey post hoc comparisons of individual means).

in situ hybridization of glans tissue from both human and rat penis revealed MC4 receptor mRNA in nerve fibers and mechanoreceptors in the glans. Glans tissue is incredibly sensitive, and direct stimulation is perceived as highly pleasurable [40]. It is therefore likely that melanocortin systems are activated simultaneously in the peripheral and central nervous systems by sexual stimuli, and help to coordinate the behavioral reaction to those stimuli. It is not yet known whether MC receptors are found on peripheral sexual tissues in females (clitoris, cervix, vagina), although the MC5 receptor has been detected in rat uterus [41].

Discussion

What do preclinical investigations of bremelanotide tell us about the potential for its use in human females? Although the sexual behavior of rats is different from that of humans, the effects of pharmacological manipulations of appetitive and consummatory sexual behaviors are strikingly similar in males of both species [1–3,5]. The similarities and differences between female rats and humans have received less attention. Reflexive behaviors in the female rat such as lordosis have no human counterpart. However, appetitive sexual responses examined in our experiments, such as solicitations, hops and darts, and even mounting by the female rat, are used to entice the male into sexual activity. These behaviors were substantially increased by bremelanotide. If these behaviors are analogous to an increase in appetitive sexual desire in human females, the potential exists for the use of bremelanotide to ameliorate various female sexual dysfunctions associated with a loss of desire.

Acknowledgments

The authors would like to thank Dr. Annette Shadiack for her initiative and help in the design of some of the experiments reported in this review. We would also like to thank Mariana Jacubovich, Shann Ménard, Tanya Van Soest, and Maric Tse, for their excellent technical assistance in conducting some of the experiments reported in this review.

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Conflict of Interest: All three authors have performed preclinical contract research for Palatin Technologies on the effect of bremelanotide in rats. Palatin is the owner of the patent for bremelanotide and is currently engaged in human clinical trials of the drug. Doctors Pfaus and Giuliani have also served as consultants to Palatin. However, all research reported in this review was conducted independently, and has been submitted to, and published by, peer-reviewed journals. None of the authors owns stock in Palatin Technologies, nor will they profit in any way from bremelanotide.

Statement of Authorship

Category 1

(a) Conception and Design
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(b) Acquisition of Data
James Pfaus; Francois Giuliano; Hélène Gelez

(c) Analysis and Interpretation of Data
James Pfaus; Francois Giuliano; Hélène Gelez

Category 2

(a) Drafting the Article
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(b) Revising It for Intellectual Content
James Pfaus; Francois Giuliano; Hélène Gelez

Category 3

(a) Final Approval of the Completed Article
James Pfaus; Francois Giuliano; Hélène Gelez

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