Drugs of the Future 2002, 27(8): 771-776

CCC: 0377-8282/2002

Review Article

Hexarelin analogues as inducers of penile erection

Antonio Argiolas*, Maria Rosaria Melis and Romano Deghenghi

Bernard B. Brodie Department of Neuroscience, University of Cagliari, 09042 Monserrato (Cagliari, Italy) and Europeptides, 95108 Argenteuil, France.
**Correspondence*

CONTENTS

Summary

Penile erection is the result of an extremely complex interaction involving the central and peripheral nervous systems which eventually leads to relaxation of the corpora cavernosa. In recent years, detailed knowledge of both the local and, to a lesser extent, central mechanisms involved has contributed to the development of numerous drugs that may be useful in the treatment of erectile dysfunction. These proerectile drugs act on various targets by different mechanisms of action and may have a positive influence on erectile dysfunction even in the presence of other complications. Hexarelin analogues are short peptides (3-7 amino acids) derived from hexarelin, a peptide originally characterized for its ability to release growth hormone. Some hexarelin analogues induce erection when injected into the paraventricular nucleus of the hypothalamus, one of the brain regions responsible for the control of erectile function, apparently through activation of central oxytocinergic neurotransmission, while others induce erection when given systemically, although to a lesser extent. As several analogues have potency comparable to that of other types of agents that induce erection by acting on the hypothalamic nucleus (*e.g.*, apomorphine, oxytocin and excitatory amino acids), hexarelin analogues may prove to be potential agents for the treatment of erectile dysfunction.

Introduction

Penile erection in mammals is the result of a complex neural central and peripheral interaction causing muscular and hemodynamic changes at the level of corpora cavernosa, corpus spongiosum and other perineal structures. The process is further complicated by peripheral and central endocrine and neuroendocrine influences, mainly due to testosterone and its metabolites (1-6 and references therein). Neural fibers carrying proerectile and antierectile information originate in the brain and travel along the spinal cord exiting in part at the level of thoraco-lumbar T4-L5 tract and in part at the level of the sacral S2-S4 tract. Fibers leaving the spinal cord at the T4-L5 level run in the hypogastric nerves, whilst those leaving the spinal cord at the S2-S4 level run in the pelvic nerves. Both hypogastric and pelvic nerves reach the pelvic plexus, the site where the cavernosal nerves originate. Cavernosal nerves innervate the corpora cavernosa, the corpus spongiosum and other perineal muscles. Fibers originating in the paravertebral sympathetic chain (L1-S1 tract) which run in the cavernosal nerves, as well as those that run in the pudendal nerves forming the dorsal nerves of the penis, should also be included among the above neural pathways. Pudendal nerves also contain penile somatosensory afferent fibers which enter the spinal cord at the S2-S4 levels, and motor fibers which leave the spinal cord at the S2-S4 level and innervate the striated bulbospongiosum and ischiocavernosum muscles localized at the base of the penis. The neural circuit formed by pelvic, cavernosal and pudendal nerves is responsible for both reflex erections, which occur after stimulation of the genitalia even when the spinal cord is lesioned above the sacral tract, and for the bulbospongiosal reflex (hyperrigidity of the penis that occurs during erection by touching the penis gland).

Neural control of penile erection

When sexual stimuli (*i.e.*, visual, auditory, olfactory, or imaginative in humans) activate the neural mechanisms which lead to penile erection, the key event is

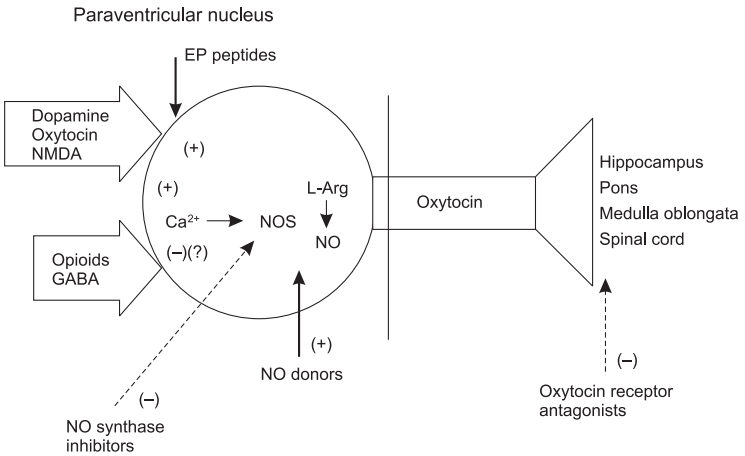

Fig. 1. Schematic representation of oxytocinergic neurons originating in the paraventricular nucleus (PVN) of the hypothalamus and projecting to extrahypothalamic brain areas and the spinal cord. Activation of these neurons by neurotransmitters and neuropeptides such as dopamine, oxytocin, excitatory amino acids, NO and hexarelin analogues, leads to penile erection, while their inhibition by GABA and opioid peptides prevents this sexual response. Activation of oxytocinergic neurons is secondary to the activation of NOS present in their cell bodies. By means of a yet to be identified mechanism, NO in turn activates oxytocin release in extrahypothalamic brain areas and in the spinal cord. These neurons are also activated when penile erection occurs in physiological contexts, such as when a male rat is in the presence of an inaccessible receptive female or during copulation.

represented by the relaxation of smooth muscles of the corpora cavernosa and the corpus spongiosum. This causes an influx of blood into the corpora cavernosa and corpus spongiosum. These, when filled, compress penile veins against the relatively indilatable tunica albuginea activating a venous-occlusive mechanism, which contributes to the maintenance of erection. Together with voluntary and/or reflex contractions of the striated bulbospongiosal and ischiocavernosal muscles, this produces the rigidity necessary for intromission (1-6 and references therein).

At penile level, 3 kinds of neural control have been characterized in detail: the first is of an adrenergic stimulatory nature, which keeps the cavernosal smooth muscles contracted rendering the penis flaccid; the second is cholinergic inhibitory type, which relaxes cavernosal smooth muscles and facilitates erection; and the third is a nonadrenergic, noncholinergic inhibitory type which facilitates erection. The latter is believed to be nitric oxide (NO), now considered as main physiological mediator of the relaxation of the corpora cavernosa although other agents may also be involved (7-9). NO is formed from L-arginine by NO synthase (NOS), which exists at penile level in 2 isoforms: neuronal which is present in neurons running in cavernosal nerves and endothelial which is present in the endothelium covering cavernosal smooth muscles. NO facilitates relaxation of cavernosal muscles by activating guanylate cyclase thus increasing cGMP concentrations (8).

Despite the detailed knowledge to date of the neural control which leads to relaxation of corpora cavernosa at local level, very little is known of the neural circuits controlling erectile function at the central level. Nevertheless, several neurotransmitters and neuropeptides that control erectile function have been identified with some certainty in the central nervous system (CNS). Among these neurotransmitters, the most widely acknowledged are dopamine, serotonin, excitatory and inhibitory amino acids and NO, while the ACTH-MSH-related peptides, oxytocin and opioid peptides are the accepted neuropeptides involved. Dopamine, excitatory amino acids, NO, ACTH-MSH peptides and oxytocin facilitate penile erection, while inhibitory amino acids (GABA) and opioid peptides inhibit this sexual response (1-3, 10-15). They influence penile erection by acting in several brain areas, which include the medial preoptic area, the paraventricular nucleus (PVN) of the hypothalamus, the hippocampus, the nucleus paragigantocellularis of the reticular formation and the spinal cord. At the paraventricular level, a group of oxytocinergic neurons that control penile erection has been identified. Activation of these neurons by dopamine receptor agonists, excitatory *N*-methyl-D-aspartic acid (NMDA) and oxytocin itself facilitates this sexual response, while their inhibition by opiate drugs and the $GABA_A$ receptor agonist muscimol prevents penile erection induced by the above compounds (Fig. 1) (2, 10-12, 16-18). Stimulation of oxytocinergic neurons mediating penile erection is apparently mediated by the activation of NOS in the PVN. Indeed, NOS inhibitors prevent dopamine receptor agonist-, NMDA- and oxytocin-induced penile erection. Classic NO donors induce penile erection when injected into the PVN by increasing oxytocinergic transmission and dopamine receptor agonists, NMDA and oxytocin increase NO production in the PVN when given at doses that induce penile erection, as shown in studies using *in vivo* microdialysis (19-22). Both responses are prevented by the NOS inhibitor N^G-nitro-L-

Table I: Structure of hexarelin analogues.

Hexarelin	His-D-Trp(2-Me)-Ala-Trp-D-Phe-LysNH_2
EP-40904	Thr-D-Trp(2-Me)-Ala-Trp-D-Phe-LysNH_2
EP-40737	D-Thr-D-Trp(2-Me)-Ala-Trp-D-Phe-LysNH_2
EP-50885	GAB-D-Trp(2-Me)-D-βNal-Phe-LysNH_2
EP-90101	GAB-D-Trp(2-Me)-D-βNal-Phe-ArgNH_2
EP-51322	GAB-D-Trp(2-Me)-D-βNal-NH_2
EP-60761	GAB-D-Trp(2-Me)-D-Trp(2-Me)-D-Trp(2-Me)-LysNH_2
EP-70555	GAB-D-Trp(2-Me)-D-Trp(2-Me)-D-Trp(2-Me)-Arg(NO_2)NH_2
EP-51216	GAB-D-Trp(2-Me)-D-Trp(2-Me)-L-Trp(2-Me)-LysNH_2
EP-80661	GAB-D-Trp(2-Me)-D-Trp(2-Me)-LysNH_2
EP-91071	GAB-D-Trp(2-Me)-LysNH_2
EP-91072	GAB-D-Trp-D-Trp-LysNH_2
EP-91073	AIB-D-Trp(2-Me)-D-Trp(2-Me)-LysNH_2

Abbreviations: GAB = γ-aminobutyryl; AIB = aminoisobutyryl; βNal = β-(2-naphthyl)-alanine

arginine methyl ester (L-NAME) injected into the PVN a few minutes prior to the above compounds (19-12). Mechanisms similar to those described above occur in oxytocinergic neurons at paraventricular level when penile erection occurs in physiological contexts, such as in the presence of a receptive female or during copulation (23-26). This suggests that oxytocinergic neurons originating in the PVN projecting to the spinal cord represent a neural pathway through which the brain centers control neural activity at penile level.

Hexarelin analogues induce penile erection

Hexarelin is a hexapeptide originally characterized for its ability to release growth hormone (GH) in laboratory animals and humans with a potency comparable to that of the natural GH-releasing hormone (GHRH). The GH-releasing effect of hexarelin, similar to that of other GH secretagogues, is mediated by the stimulation of receptors distinct from those of endogenous GHRH (27-30). This peptide also increases feeding apparently by acting on receptors different from those that release GH and that are localized mainly in the arcuate nucleus (31, 32). An endogenous ligand for these GH secretagogue receptors, namely ghrelin, has recently been isolated and characterized from the stomach of humans and rats (33).

During studies aimed at identification of the brain sites in which hexarelin acts to increase feeding and characterization of the receptors mediating this effect, we found that a group of hexarelin analogues induced episodes of penile erection indistinguishable from those induced by the dopamine receptor agonist apomorphine, oxytocin, NMDA or NO donors when injected into the PVN of male rats (34-37). Active analogues include EP-50885, EP-60761, EP-80661, EP-90101, EP-91071, EP-91072 (Table I). Some of the analogues were also able to induce penile erection when given systemically although to a lesser extent (36, 38).

The potency of some of the hexarelin analogues (*e.g.*, EP-80661, EP-60761 and EP-91072) when injected into the PVN was comparable to that reported for apomorphine, oxytocin and NMDA (36). Indeed these peptides were capable of inducing penile erection when injected into the PVN of male rats at doses as low as 20 ng. On a molar basis, the dose of these analogues that induces 50% of the maximal response corresponds to approximately 70-90 pmoles. However, the maximal effect of these analogues was lower than that observed with apomorphine and oxytocin (approximately 3.4 *versus* 4.5 erections/rat) but similar to that of NMDA (3.7 erections/rat).

Structure-activity relationship

Analysis of the amino acid sequence of the hexarelin analogues tested shows the existence of a clear structure-proerectile activity relationship (36). Indeed, the presence of a basic C-terminal amino acid (L-Lys or L-Arg) appears to be a requisite for proerectile activity, since analogues lacking such a C-terminal amino acid are inactive. When L-Arg is at the C-terminal, the presence of a NO_2 group in the guanydil moiety of the amino acid eliminates proerectile activity. The C-terminal amino acid may be preceded by one or more aromatic amino acid residues, such D-Trp(2-Me) or D-Trp or D-β-(2-naphthyl)-Ala-L-Phe. Although peptides with L-Trp close to the C-terminal basic amino acid have not been tested so far, the replacement of the D-Trp(2-Me) residue close to the C-terminal amino acid with L-Trp(2-Me), or the presence of the C-terminal tripeptide L-Trp-D-Phe-L-LysNH_2 abolishes proerectile activity. The N-terminal amino acid also seems to have a role in the proerectile activity of EP peptides. Indeed substitution of the GAB (γ-amino-butyril) group with the AIB (aminoisobutyryl) group not only abolishes proerectile activity, but also renders the analogue capable of preventing the proerectile activity of active analogues (39).

Mechanisms of action

The proerectile effect of hexarelin analogues was reduced by the oxytocin receptor antagonist [d(CH_2)$_5$Tyr(Me)^2-Orn^8]-vasotocin given into the lateral

ventricles (i.c.v.) but not into the PVN, by the NOS inhibitor L-NAME given i.c.v. or into the PVN, and by the opiate morphine, but not by the NMDA receptor antagonist dizolcipine ((+)MK-801) or by the dopamine receptor antagonist *cis*-flupenthixol all given into the PVN (35, 36). Hexarelin analogues that induce penile erection also increase NO_2- and NO_3- concentration in the paraventricular dialysate, as measured by intracerebral microdialysis (37). This suggests that the proerectile effect of hexarelin analogues is secondary to an increase in NO production in the PVN, as shown for dopamine receptor agonists, oxytocin, NMDA and NO donors (19-22). Together these results suggest that hexarelin analogues induce penile erection by activating central oxytocinergic transmission (Fig. 1). The activation of paraventricular oxytocinergic neurons controlling penile erection is apparently mediated by the stimulation of specific receptors for these hexarelin analogues possibly located in the cell bodies of the oxytocinergic neurons involved in penile erection. In fact, from the results summarized above it seems unlikely that hexarelin analogues activate oxytocinergic neurons indirectly through the release of other substances present in the PVN such as dopamine, oxytocin or excitatory amino acids which are capable of activating oxytocinergic neurons and inducing erection. Support for this hypothesis is also provided by the ability of EP-91073, a hexarelin analogue devoid of proerectile activity, to reduce penile erection induced by active analogues not only in a dose-dependent, but also a selective manner. Indeed, this hexarelin analogue is unable to reduce the proerectile effect of apomorphine, oxytocin or NMDA injected into the PVN, despite its ability to prevent the proerectile effect of active hexarelin analogues. EP-91073 also reduces the increase in paraventricular NO production that occurs concomitantly with penile erection induced by active hexarelin analogues (39).

The proerectile effect of hexarelin analogues is mediated by the stimulation of receptors different from those mediating GH release and eating behavior. Accordingly, the structure-activity relationship of hexarelin analogues for penile erection differs from that involved in GH release and eating behavior (36). Accordingly, elimination of the C-terminal L-Lys-NH_2, which eliminates proerectile activity, is unable to reduce GH release or eating behavior. Hexarelin releases GH and induces eating, but is unable to induce penile erection; EP-80661 is most effective in inducing penile erection, but is unable to release GH release and induce eating (31, 36, 37). EP-80661, unlike hexarelin, is also unable to displace labeled L-Tyr-L-Ala-hexarelin from its binding sites in rat pituitary membranes (Muccioli & Deghenghi, personal communication).

Future perspectives

Erectile dysfunction is a common problem that may significantly affect quality of life as well as psychological and social well being. The disorder is more common among the elderly and since this proportion of the population is on an increase, the prevalence of erectile dysfunction is also expected to increase. In Europe and the US epidemiological studies have shown that 10-20% of males are affected by some degree of erectile dysfunction (40-42). The considerable attention paid to this matter has increased the interest in the design and development of new agents for use in the treatment of erectile dysfunction. This suggests that the possibility of application of hexarelin analogues in the treatment of erectile dysfunction should not be left unexamined. In this regard, it is noteworthy to mention that hexarelin analogues are extremely effective in inducing penile erection when injected into the PVN of male rats and induction of erection in male rats is usually predictive of induction of erection in humans. Several analogues are also active when given systemically. However, the effect of the latter is very modest when compared to that observed after injection into the PVN. The reason for this discrepancy between systemic versus intraparaventricular administration may be due to the low amounts of hexarelin analogues reaching the CNS subsequent to systemic administration. Interestingly, downsizing of the amino acid sequence of several hexarelin analogues tested to date seems to increase the amount of EP peptide that reaches the CNS. However, this means an increased dose must be injected into the PVN in order to induce the erectile response (Fig. 2). Further studies are required in an attempt to identify a peptide with an amino acid sequence capable of inducing penile erection not only when given into the PVN but also after systemic administration. However, even if the above problem is solved, a significant amount of work will still be needed to address questions related to potency, bioavailability, half-life, toxicity and route of administration. In particular, the presence of other effects of hexarelin analogues must be ascertained prior to the testing of the same or related hexarelin analogues capable of inducing erection in humans. The latter is particularly important for those GH secretagogues, including hexarelin, that increase not only the release of GH but also ACTH and corticosteroids in several animal species including humans (27-30). Nonetheless, in spite of these factors, the development of hexarelin analogues continues to represent a viable strategy for the treatment of erectile dysfunction.

Conclusions

When injected into the PVN, hexarelin analogues induce erection in male rats by stimulating receptors different from those which mediate GH release and eating behavior following activation by hexarelin and other GH secretagogues. Stimulation of these receptors in turn elicits the activation of central oxytocinergic neurotransmission with a mechanism similar to that observed with dopamine agonists, oxytocin and excitatory amino acids. This finding deserves some comment. If hexarelin analogues indeed activate oxytocinergic neurons by stimulating specific receptors rather than by modulating the

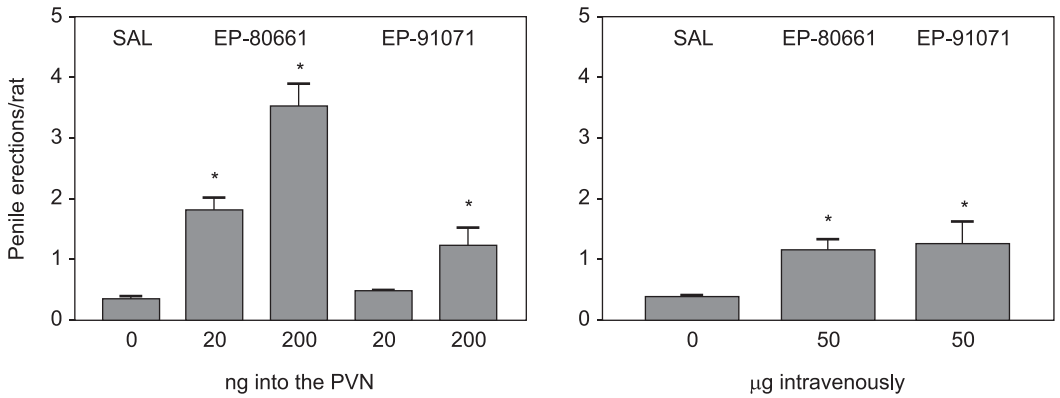

Fig. 2. Downsizing the amino acid sequence of those hexarelin analogues capable of inducing penile erection when injected into the paraventricular nucleus (PVN) requires an increase in dose for efficacy when given systemically. Indeed, EP-91071 is less effective than EP-80661 when injected into the PVN, but is as effective as EP-80661 when given i.v.

activity of other identified endogenous substances which influence erectile function at the PVN level, such receptors might represent the target of another yet to be identified endogenous substance in the PVN responsible for modulation of erection via oxytocinergic neurons. The existence of a new modulator of erection in the PVN would not to be particularly surprising, since this nucleus can be considered an integration center for the central and autonomic nervous systems in which many neurotransmitters and neuropeptides exist and often coexist to control neuroendocrine and autonomic function.

Acknowledgements

This work was partially supported by a grant from MIUR to AA.

References

1. Meisel, R.L., Sachs, B.D. *The physiology of male sexual behaviour*. In: The Physiology of Reproduction. Knobil, E., Neil, J. (Eds.). Raven Press: New York 1996, 3-96.

2. Argiolas, A., Melis, M.R. *Neuromodulation of penile erection: An overview of the role of neurotransmitters and neuropeptides*. Prog Neurobiol 1995, 47: 235-55.

3. Andersson, J.E., Wagner, G. *Physiology of erection*. Physiol Rev 1995, 75: 191-236.

4. Lue, T., Tanagho, E. *Physiology of erection and pharmacological management of impotence*. J Urol 1987, 137: 829-36.

5. De Groat, W.C., Booth, A.M. *Neural control of penile erection*. In: The Autonomic Nervous System, Maggi, C.A. (Ed.). Harwood Academic Publishers: London 1992, 465-513.

6. Dail, W.G. *Autonomic control of penile erectile tissue*. Exp Brain Res 1987, 76: 340-55.

7. Burnett, A.L., Lowenstein, C.J., Bredt, D.S., Chang, T.S.K., Snyder, S.H. *Nitric oxide: A physiological mediator of penile erection*. Science 1992, 257: 401-3.

8. Ignarro, L.J. *Biosynthesis and metabolism of endothelium-derived nitric oxide*. Ann Rev Pharmacol Toxicol 1990, 30: 535-60.

9. Rajfer, J., Aronson, W.J., Bush, P.A., Dorey, F.J., Ignarro, L.J. *Nitric oxide as a mediator of relaxation of the corpus cavernosum in response to nonadrenergic, noncholinergic neurotransmission*. N Engl J Med 1992, 326: 90-4.

10. Argiolas, A. *Neuropeptides and sexual behaviour*. Neurosci Biobehav Rev 1999, 23: 1127-42.

11. Melis, M.R., Argiolas, A. *Dopamine and sexual behavior*. Neurosci Biobehav Rev 1995, 19: 19-38.

12. Melis, M.R., Argiolas, A. *Role of central nitric oxide in the control of penile erection and yawning*. Prog Neuro-Psychopharmacol Biol Psychiatr 1997, 21: 899-922.

13. Giuliano, F., Rampin, O. *Central neural regulation of penile erection*. Neurosci Biobehav Rev 2000, 24: 517-33.

14. Heaton, J.P.W. *Central neuropharmacological agents and mechanisms of erectile dysfunction: The role of dopamine*. Neurosci Biobehav Rev 2000, 24: 561-9.

15. McKenna, K.E. *Central control of penile erection.* Int J Impotence Res 1998, 10 (Suppl. 1): 25-34.

16. Melis, M.R., Spano, M.S., Succu, S., Argiolas, A. *Activation of $GABA_A$ receptors in the paraventricular nucleus of the hypothalamus reduces apomorphine-, NMDA- and oxytocin-induced penile erection and yawning in male rats.* Neurosci Lett 2000, 281: 127-30.

17. Melis, M.R., Succu, S., Argiolas A. *Prevention by morphine of N-methyl-D-aspartic acid induced penile erection and yawning: involvement of nitric oxide*. Brain Res Bull 1997, 44: 689-94.

18. Melis, M.R., Succu, S., Argiolas. A. *Dopamine agonists increase nitric oxide production in the paraventricular nucleus of the hypothalamus: Correlation with penile erection and yawning*. Eur J Neurosci 1996, 8: 2056-63.

19. Melis, M.R., Succu, S., Iannucci, U., Argiolas, A. *Oxytocin increases nitric oxide production in the paraventricular nucleus of the hypothalamus: Correlation with penile erection and yawning*. Reg Peptides 1997, 69: 105-12.

20. Melis, M.R., Succu, S., Iannucci, U., Argiolas, A. *N-methyl-D-aspartic acid-induced penile erection and yawning: Role of hypothalamic paraventricular nitric oxide*. Eur J Pharmacol 1997, 328: 115-23.

21. Melis, M.R., Argiolas, A. *Nitric oxide donors induce penile erection and yawning when injected in the central nervous system of male rats*. Eur J Pharmacol 1995, 294: 1-9.

22. Melis, M.R., Succu, S., Mauri, A., Argiolas, A. *Nitric oxide production is increased in the paraventricular nucleus of the hypothalamus of male rats during non-contact penile erections and copulation*. Eur J Neurosci 1998, 10: 1968-74.

23. Melis, M.R., Spano, M.S., Succu, S., Argiolas, A. *The oxytocin antagonist $d(CH_2)_5Tyr(Me)^2$-Orn^8-vasotocin reduces non-contact penile erections in male rats*. Neurosci Lett 1999, 265: 171-4.

24. Melis, M.R., Succu, S., Spano, M.S., Argiolas, A. *Morphine injected into the paraventricular nucleus of the hypothalamus prevents non contact erections and impairs copulation: Involvement of nitric oxide*. Eur J Neurosci 1999, 11: 1857-64.

25. Melis, M.R., Succu, S., Mascia, M.S., Argiolas, A. *The activation of γ-aminobutyric $acid_A$ receptors in the paraventricular nucleus of the hypothalamus reduces non-contact penile erections in male rats*. Neurosci Lett 2001, 314: 123-6.

26. Melis, M.R., Argiolas, A. *Reduction of drug-induced yawning and penile erection and of noncontact erections in male rats by the activation of $GABA_A$ receptors in the paraventricular nucleus: involvement of nitric oxide*. Eur J Neurosci 2002, 15: 852-60.

27. Deghenghi, R. *Examorelin*. Drugs Fut 1996, 25: 366-8.

28. Deghenghi, R. *Growth hormone-releasing peptides*. In: Growth Hormone Secretagogues. Bercu, B.B., Walker, R.F. (Eds.) Springer: New York 1996, 85-102.

29. Muller, E.E., Locatelli, V., Cocchi, D. *Neuroendocrine control of growth hormone secretion*. Physiol Rev 1999, 79: 511-607.

30. Ghigo, E., Arvat, E., Broglio, F. et al. *Endocrine and non-endocrine activities of growth hormone secretagogues in humans*. Horm Res 1999, 51 (Suppl S3): 9-15.

31. Torsello, A., Luoni, M., Schweiger, F., Grilli, R., Guidi, M., Bresciani, E., Deghenghi, R., Muller, E.E., Locatelli, V. *Novel hexarelin analogs stimulate feeding in the rat through a mechanism not involving growth hormone release*. Eur J Pharmacol 1998, 360: 123-9.

32. Torsello, A., Locatelli, V.,Melis, M.R. et al. *Differential orexigenic effects of hexarelin and its analogues in the rat hypothalamus: Indication for multiple growth hormone receptor subtypes*. Neuroendocrinology 2000, 72: 327-32.

33. Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., Kangawa, K. *Ghrelin is a growth hormone-releasing acylated peptide from stomach*. Nature 1999, 402: 656-60.

34. Argiolas, A., Deghenghi, R. *Peptides for the treatment of erectile dysfunction*. US 6211156.

35. Melis, M.R., Spano, M.S., Succu, S. et al. *EP 60761 and EP 50885, two hexarelin analogues, induce penile erection in male rats*. Eur J Pharmacol 2000, 404: 137-43.

36. Melis, M.R., Succu, S., Spano, M.S. et al. *EP 60761- and EP 50885-induced penile erection: Structure-activity studies and comparison with apomorphine, oxytocin and N-methyl-D-aspartic acid*. Int J Impotence Res 2000, 12: 255-62.

37. Melis, M.R., Succu, S., Spano, M.R. et al. *Penile erection induced by EP 80661 and other hexarelin peptide analogues: Role of paraventricular nitric oxide*. Eur J Pharmacol 2001, 411: 305-10.

38. Giuliano, F., Argiolas, A., Bernabè, J., Mevel, K., Alexandre, L., Deghenghi, R. *Comparison of the proerectile effect of two hexarelin analogues, EP 80661 and EP 91072, with apomorphine after intravenous or intra paraventricular nucleus delivery in anesthetized rats*. Int J Impotence Res 2001, 13(Suppl. 4): S15.

39. Melis, M.S., Succu, S., Spano, M.S., Deghenghi, R., Argiolas, A. *EP 91073 prevents EP 80661-induced penile erection: New evidence for the existence of specific EP peptide receptors mediating penile erection*. Neuropharmacology 2001, 41: 254-62.

40. Virag, R., Beck-Archilly, L. *Nosology, epidemiology, clinical quantification of erectile dysfunction*. Rev Med Inter 1997, 1(Suppl. 1): 10s-13s.

41. Goldmeier, D., Keane, F.E., Carter, P. et al. *Prevalence of sexual dysfunction in heterosexual patients attending a central London genitourinary medicine clinic*. Int J STD AIDS 1997, 8: 303-6.

42. Read, S., King, M., Watson, J. *Sexual dysfunction in a primary care: Prevalence, characteristics and detection by the general practitioner*. J Public Health Med 1997, 19: 387-91.