

# Central control of penile erection: Role of the paraventricular nucleus of the hypothalamus

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## Abstract

The paraventricular nucleus of the hypothalamus is an integration centre between the central and peripheral autonomic nervous systems. It is involved in numerous functions from feeding, metabolic balance, blood pressure and heart rate, to erectile function and sexual behaviour. In particular, a group of oxytocinergic neurons originating in this nucleus and projecting to extra-hypothalamic brain areas (e.g., hippocampus, medulla oblongata and spinal cord) control penile erection in male rats. Activation of these neurons by dopamine and its agonists, excitatory amino acids (*N*-methyl-D-aspartic acid) or oxytocin itself, or by electrical stimulation leads to penile erection, while their inhibition by  $\gamma$ -amino-butyric acid (GABA) and its agonists or by opioid peptides and opiate-like drugs inhibits this sexual response. The activation of these neurons is secondary to the activation of nitric oxide synthase, which produces nitric oxide. Nitric oxide in turn causes, by a mechanism that is as yet unidentified, the release of oxytocin in extra-hypothalamic brain areas. Other compounds recently identified that facilitate penile erection by activating central oxytocinergic neurons are peptide analogues of hexarelin, a growth hormone releasing peptide, pro-VGF-derived peptides, endogenous peptides that may be released by neuronal nerve endings impinging on oxytocinergic cell bodies, SR 141716A, a cannabinoid CB1 receptor antagonist, and, less convincingly, adrenocorticotropin-melanocyte-stimulating hormone (ACTH-MSH)-related peptides. Paraventricular oxytocinergic neurons and similar mechanisms are also involved in penile erection occurring in physiological contexts, namely noncontact erections that occur in male rats in the presence of an inaccessible receptive female, and during copulation. These findings show that the paraventricular nucleus of the hypothalamus plays an important role in the control of erectile function and sexual activity. As the male rat is a model of sexual behaviour and penile physiology, which has largely increased in the last years our knowledge of peripheral and central mechanisms controlling erectile function (drugs that induce penile erection in male rats usually do so also in man), the above results may have great significance in terms of a human perspective for the treatment of erectile dysfunction.

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*Abbreviations:* ACTH, adrenocorticotropin; GABA,  $\gamma$ -amino butyric acid;  $\alpha$ -MSH,  $\alpha$ -melanocyte-stimulating hormone; NMDA, *N*-methyl-D-aspartic acid; VGF, non acronymic *vgf* gene protein

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## 1. Introduction

Penile erection is the result of a complex neural central and peripheral interaction that induces muscle and vascular changes at the level of the erectile tissues of the male genital apparatus (corpora cavernosa, corpus spongiosum, and other perineal muscles, such as the *elevator ani* muscle when present). This is further complicated by humoral and endocrine influences, exerted mainly by testosterone and its metabolites, both at central and peripheral levels (see Sachs and Meisel, 1988; Meisel and Sachs, 1994; Hull et al., 2002; and references therein). Although the achievement of penile erection is essential for the success of reproduction, this sexual response can occur not only during sexual activity but also in other contexts, such as after simple manipulation of the genitalia, or during sleep or erotic fantasies in humans, or in male rats in the presence of an inaccessible receptive female, or after treatment with several classes of drugs acting in the central nervous system or peripherally (i.e., dopamine agonists, serotonin agonists, adrenocorticotropin (ACTH)-melanocyte stimulating hormone ( $\alpha$ -MSH)-related peptides, oxytocin, hexarelin analogues, nitric oxide donors, phosphodiesterase inhibitors, soluble guanylate cyclase activators, RhoA-Rho kinase inhibitors, etc.) (see Andersson and Wagner, 1995; Argiolas and Melis, 1995, 2004; McKenna, 1998, 2000; Argiolas, 1999; Sachs, 2000; Giuliano and Rampin, 2000, 2004; Heaton, 2000; Andersson, 2001; Chitale et al., 2001; Giuliano and Allard, 2001; Melis and Argiolas, 2003). Depending on the context in which penile erection occurs, it is generally accepted that different central and peripheral neural and/or humoral endocrine mechanisms may participate in the regulation of this sexual response, often in a very complex fashion (see Fig. 1 for a schematic representation of central and peripheral neural pathways controlling penile erection). Conversely, dysfunctions of the central or peripheral autonomic nervous system, or both may impair the achievement of penile erection, even when the key effector organs (e.g., vasculature and corpora cavernosa smooth muscles) are intact. Of the central and peripheral mechanisms regulating penile erection that have been identified so far, the best known are those that control penile erection locally at the level of penile tissue, in particular those involved in the relaxation of the corpora cavernosa, the key event for the achievement of penile erection (see Andersson

and Wagner, 1995; Argiolas and Melis, 1995; Andersson, 2001; and references therein) (Table 1). This has produced in the last 10 years considerable progress in the pharmacological treatment of organic erectile dysfunction, providing

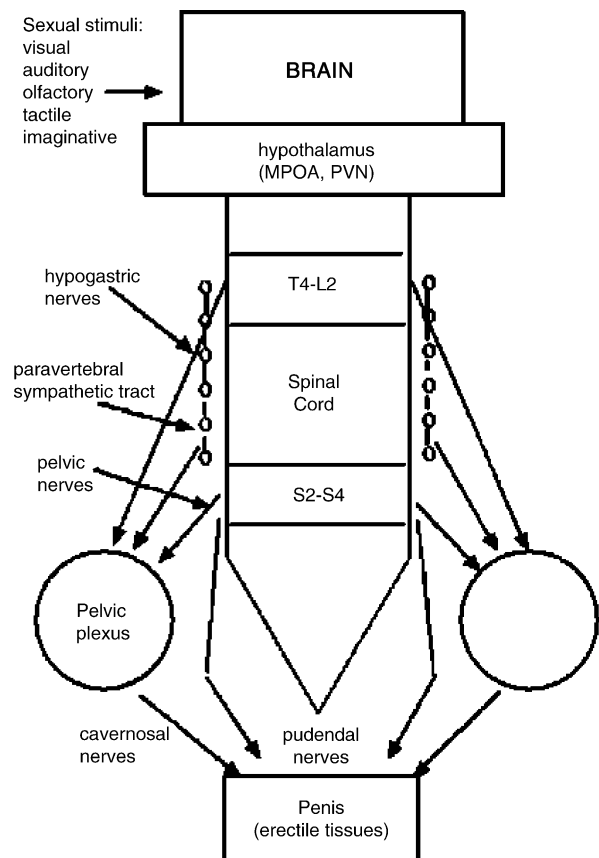


Fig. 1. Neuroanatomy of the male genital apparatus: schematic representation. When sexual stimuli reach the central nervous system, they activate neural pathways to date still unknown that are responsible for sexual activity. These travel from the brain, mainly from the hypothalamus and its nuclei (medial preoptic area and paraventricular nucleus), through the medulla oblongata and the spinal cord, to the genital apparatus. This is innervated mainly by pudendal nerves, which originate from the sacral tract of the spinal cord and contain the primary afferent sensory and motor pathways to the penis, and by cavernosal nerves, which contain the primary efferent sympathetic and parasympathetic pathways originating in the pelvic plexuses. These are innervated by hypogastric nerves, which originate in the thoracic-lumbar tract of the spinal cord, by pelvic nerves which originate in the sacral tract of the spinal cord, and by post-ganglionic fibers, which originate from the paravertebral sympathetic ganglia chain of the thoracic-lumbar tract of the spinal cord.

Table 1  
Neurotransmitters, neuropeptides and other agents that act on erectile tissues at local level

Compound	Effect on	
	Cavernosal smooth muscle	Penile vasculature
Noradrenaline	Contraction	Contraction
Acetylcholine	Relaxation <sup>a</sup>	Relaxation <sup>a</sup>
Nitric oxide	Relaxation	Relaxation
VIP (vasoactive intestinal polypeptide)	Relaxation	Relaxation
Neuropeptide Y	NA	Contraction
Endothelins	Contraction	Contraction
PGE <sub>2</sub> , PGF <sub>2α</sub> , PGD, PGI <sub>2</sub> , TXA <sub>2</sub>	Contraction	NA
PGE <sub>1</sub>	Relaxation	NA
Phosphodiesterase V inhibitors	Relaxation	None
Soluble guanylate cyclase activators	Relaxation	Relaxation
RhoA-Rho kinase inhibitors	Relaxation	Relaxation

Information on the mechanism of action of the above compounds can be found in the cited references. *Abbreviations:* PG, prostaglandins; TX, tromboxane; NA, not available.

<sup>a</sup> The relaxing effect of acetylcholine in these tissue is indirect, e.g., mediated by the release of nitric oxide from endothelial cells laying over smooth muscle cells. Indeed, acetylcholine usually contracts all smooth muscle fibres studied so far by acting on muscarinic receptors.

drugs capable of interacting, often selectively, with the neurotransmitters and/or modulators that control the relaxation of corpora cavernosa (see Garcia-Reboll et al., 1997; Moreland et al., 2001; Lue et al., 2004). In contrast, much less is known of the mechanisms through which the central nervous system controls penile erection, although some progress in this field has also been achieved recently. Indeed, at the central level several neurotransmitters and neuropeptides, which control erectile function, have also been identified (Table 2). These compounds act in several brain areas conveying information to the lower genital apparatus through the spinal cord. Among these areas, the most studied are the medial preoptic area (Hull et al., 1986, 1989, 1995; Sato et al., 1995, 2001; Sato and Christ, 2000; Giuliano et al., 1996; Liu et al., 1997a; Matuszewich et al., 2000), the paraventricular nucleus of the hypothalamus (Melis et al., 1986, 1987, 1998, 1999b, 2002; Eaton et al., 1991; Liu et al., 1997b), the amygdala (Kondo et al., 1998;

Dominguez et al., 2001), the nucleus paragigantocellularis of the ventral reticular formation (Marson and McKenna, 1990, 1992, 1994; Marson et al., 1992, 1993) and the spinal cord (Wagner and Clemens, 1991, 1993; Marson and McKenna, 1996; Rampin et al., 1997; Tang et al., 1998; Bancila et al., 1999; Veronneau-Longueville et al., 1999; Giuliano et al., 2001a, 2001c, 2002; Giuliano and Allard, 2001). The aim of this short review is to summarize the results obtained independently by several groups of researchers with different experimental (pharmacological, electrophysiological and neuroanatomical) approaches that show an important role of the paraventricular nucleus of the hypothalamus in the control of erectile function. Two examples are (1) electrical or chemical stimulation of this hypothalamic nucleus induces penile erection as revealed either by visual inspection or by telemetry monitoring of the intracavernosal pressure in awake or anesthetized male rats (Roeling et al., 1991; Melis et al., 1994a; Chen et al., 1997;

Table 2  
Neurotransmitters and neuropeptides that influence penile erection in the central nervous system

Compound	Effect on		Brain area involved
	Erection	Penile reflexes	
Dopamine	Facilitatory	Facilitatory	PVN, MPOA
Serotonin	Inhibitory, facilitatory <sup>a</sup>	Inhibitory	SpC, MPOA
Excitatory amino acids	Facilitatory	NA	PVN
GABA	Inhibitory	Inhibitory	PVN, SpC
Nitric oxide	Facilitatory	NA	PVN, MPOA
Acetylcholine	Facilitatory	NA	Hippocampus
Noradrenaline	Inhibitory	NA	Hypothalamus
Oxytocin	Facilitatory	NA	PVN, SpC, Hippocampus
ACTH-MSH	Facilitatory	NA	APVH
Opioid peptides	Inhibitory	NA	PVN, MPOA
Hexarelin peptides	Facilitatory	NA	PVN
Pro-VGF peptides	Facilitatory	NA	PVN
Endocannabinoids	Inhibitory <sup>a</sup>	NA	PVN

Information on the mechanism of action of the above compounds can be found in the cited References. *Abbreviations:* PVN, paraventricular nucleus of the hypothalamus; MPOA, medial preoptic arera; SpC, spinal cord; APVH, periventricular hypothalamic area. NA, not available.

<sup>a</sup> Depending on the receptor subtype involved.

Zahran et al., 2000; Chen and Chang, 2003), and (2) its bilateral lesions reduce dramatically the proerectile effect of several compounds (Argiolas et al., 1987a; Liu et al., 1997b), as well as erections that occur in physiological contexts, for example, noncontact erections seen in male rats in the presence of an inaccessible receptive female (Liu et al., 1997b), which are induced mainly by physiological olfactory sexual stimuli (Sachs et al., 1994; Sachs, 1997; Edwards and Davis, 1997), or erections during copulation (Liu et al., 1997b). Together these studies show that a group of oxytocinergic neurons originating in the paraventricular nucleus of the hypothalamus and projecting to several extra-hypothalamic brain areas (e.g., hippocampus, pons, medulla oblongata and spinal cord) control penile erection in male rats. As shown in detail below, activation of these neurons by several endogenous (e.g., classical neurotransmitters and/or neuropeptides) and exogenous substances induces penile erection, while their inhibition reduces this sexual response (Fig. 2). As the literature on these studies has already been reviewed in detail (Andersson and Wagner, 1995; Argiolas and Melis, 1995; McKenna, 1998; Argiolas, 1999; Andersson, 2001; Melis and Argiolas, 2003; Argiolas and Melis, 2004), particular attention is dedicated to the most recent findings confirming such a role. As the male rat is a well recognized model in the field of sexual behaviour and penile physiology, which has largely contributed in the last 15 years to our knowledge of the peripheral and central mechanisms controlling erectile function and sexual behaviour (drugs that induce penile erection in male rats usually do so also in man), the reviewed studies have great significance in a human perspective for the treatment of erectile dysfunction.

## 2. Paraventricular oxytocinergic neurons projecting to extra-hypothalamic brain areas and to the spinal cord control penile erection

The paraventricular nucleus of the hypothalamus is considered a sort of integration centre between the central and peripheral autonomic nervous systems. In line with this role, it is characterized by a very complex architecture and is involved in numerous functions, from stress to the control of feeding, body energy balance, blood pressure, heart rate and sexual activity, including penile erection (see Swanson and Sawchenko, 1983). It also contains the cell bodies of oxytocin and vasopressin neurons that project to the neurohypophysis, from which the neuropeptides are released in several circumstances, and whose hormonal roles in parturition and lactation and in water and electrolyte control, respectively, have been, and are still extensively investigated at various levels (see Amico and Robinson, 1985; Ivell and Russel, 1995). In addition to neurohypophyseal neurons, oxytocin and vasopressin are also present in neurons that project from the paraventricular nucleus and surrounding structures to extra-hypothalamic brain areas and the spinal cord (see Buijs, 1978; Sofroniew, 1983). These central oxytocinergic and vasopressinergic neurons are thought to be involved in different central functions, such as memory, learning, affiliative and socio-sexual behaviors (see Argiolas and Gessa, 1991; Richard et al., 1991; Pedersen et al., 1992; Carter et al., 1997). In particular, a group of these oxytocinergic neurons seems to play an important role in the control of penile erection and copulatory behaviour (see Argiolas and Melis, 1995, 2004; Melis and Argiolas, 2003). This involvement was originally suggested by the discovery

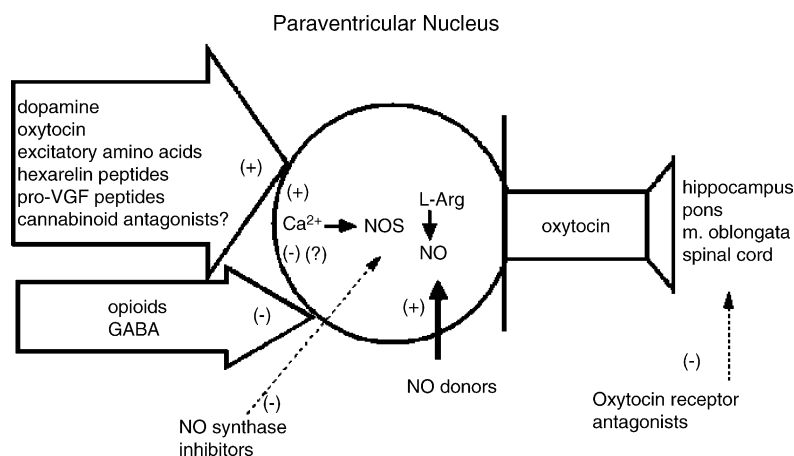


Fig. 2. Schematic representation of oxytocinergic neurons, which originate in the paraventricular nucleus of the hypothalamus and project to extra-hypothalamic brain areas and the spinal cord. The activation of these neurons by dopamine, excitatory amino acids, oxytocin itself, hexarelin analogue peptides, and pro-VGF-derived peptides or by the blockade of cannabinoid CB1 receptors leads to penile erection, which can be reduced and/or abolished by the stimulation of opioid and GABAergic receptors. The activation of oxytocinergic neurons is secondary to the activation of nitric oxide-synthase present in these neurons. Indeed endogenous nitric oxide formed by the stimulation of dopamine, excitatory amino acid or oxytocin receptors or exogenous nitric oxide, as that derived from nitric oxide donors given directly into the paraventricular nucleus, activates oxytocinergic neurons by an yet unidentified mechanism. This causes in turn the release of oxytocin in brain areas distant from the paraventricular nucleus inducing penile erection. Mechanisms similar to those described above also operate when penile erection occurs in physiological contexts, namely as when male rats are placed in the presence of an inaccessible receptive female or during copulation.

that oxytocin induces penile erection in rats (Argiolas et al., 1985, 1986), a finding soon followed by identification of the paraventricular nucleus of the hypothalamus as one of the most sensitive brain areas for the proerectile effect, not only of oxytocin, but also of many other agents, some of which were already known to facilitate penile erection and sexual behaviour, i.e., apomorphine, a classical dopamine receptor agonist (Melis et al., 1986, 1987).

Since then, pharmacological, electrophysiological and immunocytochemical studies have revealed that oxytocinergic neurons projecting from the paraventricular nucleus to the hippocampus, the medulla oblongata and to the spinal cord play a key role in controlling penile erection (see Sachs and Meisel, 1988; Andersson and Wagner, 1995; Argiolas and Melis, 1995; McKenna, 1998, 2000; Giuliano and Rampin, 2000). Of particular importance in penile erection are oxytocinergic neurons that project to the spinal cord. Indeed these neurons project to the lumbar-sacral region of the spinal cord to make synapses with neurons that run in the hypogastric and pelvic nerves and in the paravertebral thoracolumbar sympathetic chain to the pelvic plexus (see Janig and McLachlan, 1987; Sachs and Meisel, 1988; Meisel and Sachs, 1994; Andersson and Wagner, 1995; Argiolas and Melis, 1995; McKenna, 1998; Hull et al., 2002) and from this through the cavernosal nerves directly to the corpora cavernosa of the penis (Wagner and Clemens, 1991, 1993; Argiolas and Melis, 1995; Chen et al., 1997; McKenna, 1998; Tang et al., 1998; Argiolas, 1999; Bancila et al., 1999; Veronneau-Longueville et al., 1999; Giuliano and Allard, 2001; Melis and Argiolas, 2003). These neurons are perhaps the only descending pathways by means of which the central nervous system influences erectile function to have been identified with any degree of certainty to date (Wagner and Clemens, 1991, 1993; Tang et al., 1998; Bancila et al., 1999; Veronneau-Longueville et al., 1999). In addition, a serotonergic pathway that originates in the nucleus paragigantocellularis exerts an inhibitory control on penile reflexes and ejaculation (Marson and McKenna, 1990, 1992; Marson et al., 1992, 1993). Other oxytocinergic neurons, for instance those projecting to the hippocampus and to the ventral medulla, more precisely in the region containing the nucleus paragigantocellularis, seem also to be involved (see Melis et al., 1987, 1992a; Chen et al., 1992; Bancila et al., 2002). As recalled above several lines of experimental evidence suggest that these neurons, when activated, release oxytocin and facilitate penile erection (and copulatory behavior), while their inhibition reduces the sexual response.

### **3. Neurotransmitters and neuropeptides that activate paraventricular oxytocinergic neurons facilitate penile erection**

As reviewed in detail in the following sections, the endogenous neurotransmitters and neuropeptides that activate oxytocinergic neurons at the paraventricular level

and induce penile erection identified so far are dopamine, excitatory amino acids, nitric oxide and oxytocin itself (see also Melis and Argiolas, 2003; and references therein). With the exception of nitric oxide, which is likely to act as an intracellular messenger inside the cell bodies of oxytocinergic neurons (see below), in general all these neuromediators apparently act by stimulating specific receptors located on the cell bodies of oxytocinergic neurons, although indirect effects cannot be always ruled out. This, in turn, causes the release of oxytocin in sites distant from the paraventricular nucleus, e.g., the hippocampus, medulla oblongata and spinal cord. The majority of exogenous substances that induce penile erection when injected into the paraventricular nucleus mimic the action of the above reported endogenous neurotransmitters, acting as agonists of the receptors for these compounds.

Hexarelin analogue peptides, VGF peptides and the cannabinoid CB1 antagonist SR 141716A have been added recently to the list of compounds that induce penile erection when injected into the paraventricular nucleus by activating oxytocinergic neurotransmission. Hexarelin analogue peptides derive from hexarelin, a synthetic peptide originally discovered for its ability to induce growth hormone release in laboratory animals and in humans, with potency comparable to that of authentic growth hormone releasing hormone (GH-RH) (Deghenghi, 1996; Deghenghi et al., 1994; Muller et al., 1999). Apparently these peptides also act to facilitate penile erection by stimulating specific receptors located on the cell bodies of oxytocinergic neurons (Melis et al., 2001a). The cannabinoid antagonist of the CB1 receptor SR 141716A also facilitates penile erection by activating oxytocinergic neurotransmission at the paraventricular level, but its exact mechanism of action has still to be elucidated (Melis et al., 2004a). VGF peptides derive from VGF, a protein originally discovered for its rapid expression after NGF stimulation in several tissues including the brain and the paraventricular nucleus, and are apparently endogenously produced by VGF proteolytic cleavage (see Trani et al., 2002). The results available so far suggest that VGF peptides also activate oxytocinergic neurotransmission (see below). Finally, some evidence was also obtained that ACTH,  $\alpha$ -MSH and related peptides induce penile erection by increasing oxytocinergic neurotransmission (see Martin and McIntyre, 2004; and references therein), although definitive evidence is not obtained yet (see below).

#### *3.1. Dopamine and dopamine receptor agonists*

The facilitatory effect of dopamine on penile erection and sexual behaviour was first discovered in 1970, when drugs that facilitate dopaminergic transmission (i.e., L-DOPA, dopamine receptor agonists) were found to be able to induce penile erection and increase libido in man (see Melis and Argiolas, 1995a; and references therein). These findings were followed soon by experimental data in laboratory animals, mainly rodents, which showed that dopamine

receptor agonists, such as apomorphine, bromocriptine, lisuride and others induce penile erection. Apomorphine, a mixed D1/D2 receptor agonist, was the first dopaminergic agonist found to be capable of inducing penile erection when injected into the paraventricular nucleus of male rats (Melis et al., 1987). This effect was apparently mediated by the stimulation of D2 receptors, since it was mimicked by selective D2 agonists, such as LY 171555, but not by D1 agonists, such as SKF 38393, and it was prevented by selective D2 receptor antagonists, such as L-sulpiride or haloperidol (Melis et al., 1987). The ability of apomorphine to induce penile erection when injected into the paraventricular nucleus was also confirmed by telemetry studies showing that the dopamine agonist given into the paraventricular nucleus is able to increase intracavernosal pressure in awake male rats without modifying systemic blood pressure (Giuliano et al., 2001b; Chen et al., 1999), as found after systemic injection (Bernabè et al., 1999). These studies also confirmed a main role of D2 receptors, as D1 agonists were found to be unable to increase intracavernosal pressure when injected into the paraventricular nucleus (Chen et al., 1999).

Several lines of experimental evidence suggest that paraventricular D2 receptors, whose stimulation induces penile erection, are located on the cell bodies of oxytocinergic neurons. First, the paraventricular nucleus contains dopaminergic nerve terminals that belong to the so-called incertohypothalamic dopaminergic neurons. The cell bodies of these neurons are situated in the A13 and A14 group of Dahlstrom and Fuxe, (1964), arborize extensively and innervate several hypothalamic structures, including paraventricular oxytocinergic neurons projecting to the neurohypophysis and/or to extra-hypothalamic brain areas (Buijs et al., 1984; Lindvall et al., 1984). Second, apomorphine increases not only oxytocin concentration in plasma of rats and monkeys (Melis et al., 1989a; Cameron et al., 1992), but also oxytocin content in extra-hypothalamic brain areas, such as the hippocampus, at doses that induce penile erection (Melis et al., 1990). Moreover, various studies show that penile erection induced by the stimulation of paraventricular D2 receptors is mediated by oxytocin released in these areas. First, bilateral electrolytic lesion of the paraventricular nucleus, which almost completely eliminates oxytocin from extra-hypothalamic brain areas (Hawthorn et al., 1985), abolishes apomorphine-induced penile erection (Argiolas et al., 1987a). Second, selective oxytocin receptor antagonists given into the lateral ventricles, but not into the paraventricular nucleus, reduce dose-dependently apomorphine-induced penile erection with a potency that is parallel to that of these compounds in blocking oxytocin receptors (Melis et al., 1989b). Finally, oxytocin receptor antagonists given into the lateral ventricles also prevent the facilitatory effect of apomorphine on male rat sexual behaviour (Argiolas et al., 1989a).

The mechanism through which D2 receptors activated by dopamine or by dopamine agonists increase the activity of

oxytocinergic neurons, and in turn the release of oxytocin from these neurons in extra-hypothalamic brain areas, is still unclear. Different experimental results suggest that the stimulation of D2 receptors causes an increase in intracellular  $Ca^{2+}$  ions, which leads to the activation of nitric oxide-synthase, a  $Ca^{2+}$ -calmodulin-dependent enzyme. The increased nitric oxide production causes in turn the activation of oxytocinergic neurons. First, apomorphine-induced penile erection is prevented by organic calcium channel blockers and  $\omega$ -conotoxin GVIA, a potent and selective blocker of voltage-dependent  $Ca^{2+}$  channels of the N-type (McCleskey et al., 1987), given into the paraventricular nucleus (see Argiolas et al., 1990; and references therein). Second, apomorphine-induced penile erection is prevented by  $N^G$ -nitro-L-arginine methylester, a potent nitric oxide-synthase inhibitor given into the paraventricular nucleus (Melis et al., 1994b). Third, apomorphine and other D2 agonists given at doses that induce penile erection increase nitric oxide production in the paraventricular dialysate obtained by intracerebral microdialysis, an increase that is prevented by inhibition of paraventricular nitric oxide-synthase (Melis et al., 1996). The mechanism by means of which nitric oxide produced by the activation of D2 receptors activates oxytocinergic neurons is still unknown, although available data suggest that nitric oxide acts as an intracellular mediator and that guanylate cyclase is not involved (see Section 3.3).

The above explanation has been often considered not very convincing, mainly because the stimulation of dopamine D2 receptors is usually coupled to inhibition rather than excitation of the cell bodies of the neurons containing these receptors through different G protein coupled mechanisms (see Sokoloff and Schwartz, 1995). Indeed this raises the possibility that the excitatory effect of dopamine on oxytocinergic neurons mediating penile erection is indirect rather than direct, e.g., mediated by changes in the activity of other compounds that modulate oxytocinergic activity in the paraventricular nucleus. However, a possible explanation for this discrepancy, which is in line with a direct stimulation of paraventricular oxytocinergic neurons by dopamine, has been suggested recently by the discovery of a G protein-coupled dopamine D2 receptor subtype, the stimulation of which causes an increase in  $Ca^{2+}$  influx in cell preparations containing a cloned version of this receptor subtype (Moreland et al., 2004). This dopamine D2 receptor subtype is a D4 receptor, and a recently discovered selective agonist (e.g., ABT 724) (*N*-methyl-4-(2-cyanophenyl)piperazynil-3methylbenzamide maleate) was found capable of inducing penile erection in male rats when given systemically (Brioni et al., 2004). This effect was not found with the selective dopamine D2 receptor subtype agonist PNU-95666E (R-5,6-dihydro-*N,N*-dimethyl-4H-imidazo[4,5,1-*i*]quinolin-5-amine) (Hsieh et al., 2004), which was also unable to increase  $Ca^{2+}$  influx (Brioni et al., 2004; Moreland et al., 2004). In line with the above hypothesis and with the above findings, PD 168,077 (*N*-methyl-4-(2-cyanophenyl)piperazynil-3methyl-

benzamide maleate), another selective dopamine D4 agonist (Heier et al., 1997), induces penile erection when injected into the paraventricular nucleus, although less effectively than apomorphine. The proerectile effect of PD 168,077 is prevented by L-745,870 (3-(4-[chlorophenyl] piperazin-1-yl)-methyl-1H-pyrrolo[2,3-B]pyridine trihydrochloride), a selective antagonist of D4 receptors (Patel et al., 1997) (Fig. 3) (Melis et al., 2005). The proerectile effect of PD 168,077 is also reduced by  $N^G$ -nitro-L-arginine methylester, a potent inhibitor of nitric oxide-synthase, given into the paraventricular nucleus and by  $d(CH_2)_5Tyr(Me)^2$ -Orn<sup>8</sup>-vasotocin, a selective oxytocin receptor antagonist given

into the lateral ventricles but not in the paraventricular nucleus, in line with the hypothesis that this dopamine D4 agonist also activates oxytocinergic neurons by activating nitric oxide-synthase, to release oxytocin in extra-hypothalamic brain areas, which in turn facilitate penile erection (Fig. 3) (Melis et al., 2005). These new findings are in line with the hypothesis that dopamine can influence penile erection by acting on D4 receptors possibly located on the cell bodies of paraventricular oxytocinergic neurons, and which cause an increased  $Ca^{2+}$  influx into the cell bodies of oxytocinergic neurons. However, further experiments with selective agonists of the other D2 receptor subtypes (mainly D2 and D3) are necessary to identify the exact role of all these receptors in the control of erectile function at the paraventricular level. In this regard, it is important to note that apomorphine, which acts potently on all dopamine receptor subtypes (see Brioni et al., 2004; and references therein), is much more effective than PD 168,077 in inducing penile erection when injected into the paraventricular nucleus. This might be explained by a higher affinity of apomorphine on dopamine D4 receptors when compared to PD-168077, as suggested by in vitro studies showing that the EC<sub>50</sub> of apomorphine (1.2 nM) is lower than that of PD-168,077 (5.5 nM) in increasing the GTP $\gamma$ S binding activity. This is a measure of the activation of G-protein coupled receptors after receptor binding, used to study the activation of dopamine D4 receptors (see Hsieh et al., 2004; and references therein). Alternatively, PD 168,077 may act as a dopamine D4-receptor partial agonist, or the concomitant activation of different dopamine receptor subtypes by apomorphine may produce a higher activation of oxytocinergic neurons mediating penile erection than the activation by PD 168,077 of the dopamine D4 receptor subtype only.

The involvement of endogenous dopamine at the paraventricular level in the control of penile erection occurring in physiological contexts has been recently supported by microdialysis studies in male rats showing noncontact erections in the presence of an inaccessible receptive female and during copulation. Indeed the concentrations of dopamine and 3,4-dihydroxy-phenylacetic acid (DOPAC), its main metabolite, were found to be increased in the extra-cellular dialysate obtained from the paraventricular nucleus of sexually potent male rats showing noncontact erections when in the presence of an inaccessible ovariectomized estrogen + progesterone treated receptive female (Melis et al., 2003). The increase of the dopamine and DOPAC concentrations was even higher when copulation with the receptive female was allowed (Melis et al., 2003). These findings support the hypothesis that dopamine neurotransmission is increased in the paraventricular nucleus during penile erection occurring in physiological contexts, as noncontact erections and copulation, as found in the medial preoptic area (Hull et al., 1995). These results are in contrast with early studies aimed to find evidence supporting such a role of dopamine in the paraventricular nucleus. In one of these studies, the injection into the

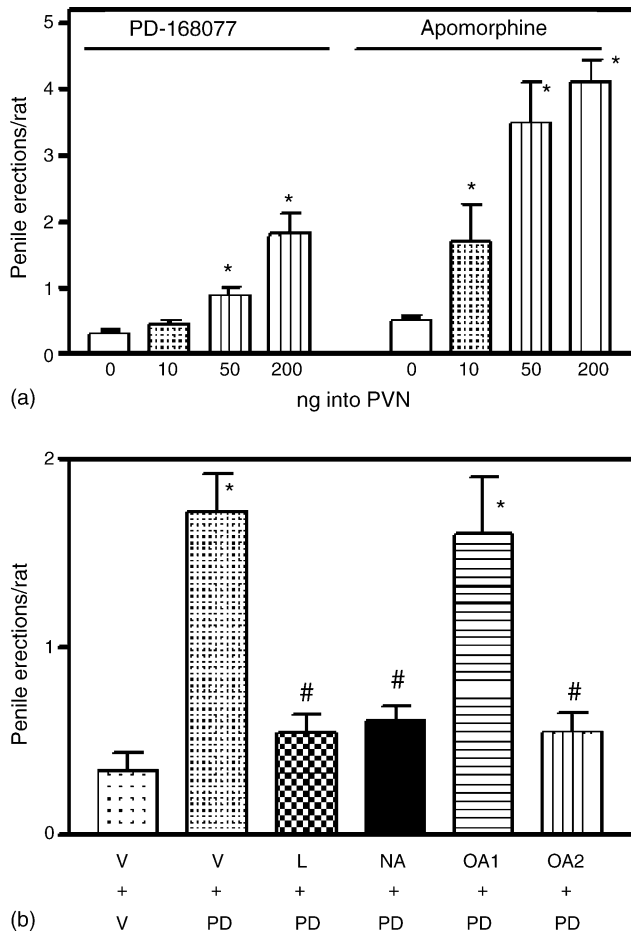


Fig. 3. PD 168,077 injected into the paraventricular nucleus induces penile erection: comparison with apomorphine (a) and effect of the prior treatment with L-745,870, a D4 receptor antagonist, or  $d(CH_2)_5$ -Tyr(Me)-Orn<sup>8</sup>-vasotocin, an oxytocin receptor antagonist, or  $N^G$ -nitro-L-arginine methylester (L-NAME), an inhibitor of nitric oxide-synthase. PD 168,077 or apomorphine (0, 50 and 200 ng) were injected into the paraventricular nucleus in a volume of 0.3  $\mu$ l in 2 min of vehicle (DMSO for PD 168,077 and saline for apomorphine). L-745,870 (1  $\mu$ g)(L),  $d(CH_2)_5$ -Tyr(Me)-Orn<sup>8</sup>-vasotocin (1  $\mu$ g)(OA1) and L-NAME (25  $\mu$ g)(N) were injected into the paraventricular nucleus in a volume of 0.3  $\mu$ l in 2 min of vehicle (saline for all three compounds) 15 min before PD 168,077 (200 ng)(PD).  $d(CH_2)_5$ -Tyr(Me)-Orn<sup>8</sup>-vasotocin (1  $\mu$ g)(OA2) was also injected into the lateral ventricles 15 min before PD 168,077. Values are means  $\pm$  S.E.M. of seven rats per group. \* $P < 0.01$  with respect to control rats; # $P < 0.01$  with respect to the corresponding group treated with PD 168,077 alone (one-way ANOVA).

paraventricular nucleus of dopamine receptor antagonists was found to be unable to reduce noncontact erections, despite the ability of these compounds to prevent dopamine agonist-induced penile erection (Melis et al., 2000a). One possible explanation for this discrepancy is that in the latter study, dopamine receptor antagonists were injected unilaterally and not bilaterally into the paraventricular nucleus, and this would have produced only a partial blockade of dopaminergic receptors throughout the entire nucleus, insufficient to prevent the sexual response induced by female olfactory cues, which are likely to activate dopamine activity in both sides of the hypothalamic nucleus.

### 3.2. Excitatory amino acid receptor agonists

The paraventricular nucleus of the hypothalamus is very rich in synapses containing an excitatory amino acid as a neurotransmitter (e.g., glutamic acid and aspartic acid) (Van Den Pol, 1991). Nevertheless, the facilitatory action of excitatory amino acids in the paraventricular nucleus of the hypothalamus on penile erection was discovered only recently (Roeling et al., 1991; Melis et al., 1994a). Accordingly-methyl-D-aspartic acid (NMDA), a selective agonist of the NMDA receptor subtype, but not ( $\pm$ )- $\alpha$ -amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA), a selective agonist of the AMPA receptor subtype, or ( $\pm$ )-trans(1)-amino-1,3-cyclopentane dicarboxylic acid (ACPD), a selective agonist of the metabotropic receptor subtype, was found capable of inducing penile erection when injected into the paraventricular nucleus of freely moving rats (Melis et al., 1994a). The NMDA effect was prevented by NMDA receptor antagonists, such as MK-801 (Wong et al., 1988). More recently, in telemetry studies aimed at monitoring intracavernosal pressure, NMDA was found more active than agonists of the other excitatory amino acid receptor subtypes when injected into the paraventricular nucleus in increasing intracavernosal pressure in awake or anaesthetized male rats (Zahran et al., 2000; Chen and Chang, 2003).

It is likely that NMDA receptors mediating penile erection are probably located on the cell bodies of oxytocinergic neurons, as excitatory amino nerve endings impinge on oxytocinergic cell bodies in the paraventricular nucleus (Van Den Pol, 1991). Accordingly, the proerectile effect of NMDA is apparently mediated by the activation of oxytocinergic neurotransmission, being abolished by  $d(\text{CH}_2)_5\text{Tyr}(\text{Me})^2\text{-Orn}^8\text{-vasotocin}$ , a potent and selective oxytocin receptor antagonist, given into the lateral ventricles, but not into the paraventricular nucleus (Melis et al., 1994a). The NMDA-induced activation of oxytocinergic transmission seems also secondary to the activation of nitric oxide-synthase, since NMDA-induced penile erection is prevented by the inhibition of paraventricular nitric oxide-synthase (Melis et al., 1994c), and NMDA injected into the paraventricular nucleus at doses that induce penile erection increases nitric oxide production in the hypothalamic

nucleus (Melis et al., 1997a). The NMDA activation of nitric oxide-synthase may also be secondary to an increased  $\text{Ca}^{2+}$  influx in oxytocinergic cell bodies through the  $\text{Ca}^{2+}$  channel-coupled NMDA receptors, as shown in several neural preparations (see Snyder, 1992, for a review). Nitric oxide in turn activates oxytocinergic transmission releasing oxytocin in extra-hypothalamic areas involved in the control of erectile function (see below). Unfortunately, the origin of glutamatergic projections that activate paraventricular oxytocinergic neurons mediating penile erection is unknown, although some neuroanatomical and electrophysiological evidence suggest that they may originate in the hippocampus (Chen et al., 1992; Saphier and Feldman, 1987). However, intra-paraventricular and intra-hypothalamic glutamatergic neurons projecting to the PVN have also been identified (Csaki et al., 2000; and references therein). Irrespective of their site of origin, glutamatergic projections to the PVN are apparently involved in the expression of noncontact erections, which occur in male rats in the presence of an inaccessible receptive female (Melis et al., 2000a), and which are also mediated by the activation of central oxytocinergic transmission (Melis et al., 1999a) (see also Section 1). Indeed, noncontact erections are reduced by blockade of NMDA receptors in the paraventricular nucleus, this reduction being followed by a decrease in the increased nitric oxide production that occurs in the hypothalamic nucleus in this physiological context (Melis et al., 2000a).

The involvement of endogenous excitatory amino acids at the paraventricular level in the control of penile erection occurring in physiological contexts has been confirmed recently by microdialysis studies in male rats showing noncontact erections in the presence of an inaccessible receptive female and during copulation. Indeed the concentrations of both glutamic acid and aspartic acid were found to be increased in the extra-cellular dialysate obtained from the paraventricular nucleus of sexually potent male rats showing noncontact erections (Melis et al., 2004b). Such increases were found even higher when copulation with the receptive female was allowed (Melis et al., 2004b). Together with the ability of the blockade of paraventricular NMDA receptors to reduce noncontact erections recalled above, these findings provide strong support to the hypothesis that excitatory amino acid neurotransmission is also increased in the paraventricular nucleus during penile erection occurring in physiological sexual contexts.

### 3.3. Nitric oxide

The involvement of paraventricular nitric oxide in the control of penile erection was first suggested by the ability of nitric oxide synthase inhibitors given into the hypothalamic nucleus to prevent penile erection induced by dopamine agonists, NMDA and oxytocin (Melis and Argiolas, 1993; Melis et al., 1994b,c). In this regard, it is pertinent to recall that the paraventricular nucleus of the hypothalamus is one of the richest brain areas in nitric oxide-synthase and that the

enzyme is present on the cell bodies of oxytocinergic neurons (see [Bredt and Snyder, 1990](#); [Vincent and Kimura, 1992](#); [Torres et al., 1993](#); [Sanchez et al., 1994](#); [Sato-Suzuki et al., 1998](#)). This was soon confirmed by other studies showing that classic nitric oxide donors (e.g., nitroglycerin, sodium nitroprusside, isoamyl-nitrite, hydroxylamine, etc.), but also high doses of L-arginine, injected into the paraventricular nucleus induce penile erection episodes indistinguishable from those seen after dopamine agonists, NMDA and oxytocin ([Argiolas, 1994](#); [Melis et al., 1995](#); [Melis and Argiolas, 1995b](#)), but see also ([Sato et al., 1999](#)). The mechanism by means of which these compounds induce penile erection is apparently secondary to the release of nitric oxide, which causes in turn the activation of oxytocinergic neurons. Indeed, their proerectile effect is prevented by the injection of oxytocin antagonists into the lateral ventricles, which block central oxytocinergic receptors, as found for dopamine agonist-, oxytocin- and NMDA-induced penile erection (see [Argiolas and Melis, 1995](#); [Melis and Argiolas, 1997, 2003](#); [Chen and Chang, 2002](#)). As recalled above, the mechanism by means of which endogenous or exogenous nitric oxide activates oxytocinergic neurons to release oxytocin in brain areas distant from the paraventricular nucleus to facilitate penile erection is still unknown. In particular, available data suggest that guanylate cyclase, the best known target of nitric oxide ([Bredt and Snyder, 1990](#); [Southam and Garthwaite, 1993](#); [Schuman and Madison, 1994](#)), is apparently not involved, as the injection of the stable phosphodiesterase-resistant cGMP analogue 8Br-cGMP into the paraventricular nucleus is unable to induce penile erection in male rats ([Melis and Argiolas, 1995b, 1997](#)). To this regard, it is noteworthy that immunocytochemical studies failed to identify appreciable amounts of guanylate cyclase in the paraventricular nucleus ([Southam and Garthwaite, 1993](#); [Torres et al., 1993](#)), but see also ([Vacher et al., 2003](#)). However, this does not rule out the possibility that the enzyme might be involved in the control of erectile function in other brain areas. Accordingly, the injection into the lateral ventricles, but not into the paraventricular nucleus, of drugs that interfere with guanylate cyclase (e.g., methylene blue or LY 83583) does indeed prevent penile erection induced by dopamine agonists, oxytocin or NMDA ([Melis et al., 1994b, 1995](#); [Melis and Argiolas, 1995b](#)). Although the mechanism of action of nitric oxide in the paraventricular nucleus is unknown, experimental evidence suggests that this molecule acts as an intracellular messenger inside the neurons in which it is produced (e.g., oxytocinergic cell bodies), rather than as an intercellular messenger. This is suggested mainly by microdialysis studies, which show that penile erection induced by a dopamine agonist, oxytocin or NMDA is not prevented by hemoglobin, a potent nitric oxide scavenger, in spite of the ability of this compound to prevent the increase in the concentration of nitric oxide, which occurs concomitantly with penile erection ([Melis et al., 1996, 1997a,b](#)). In fact this apparently paradoxical result may be

explained simply by assuming that hemoglobin reduces nitric oxide concentration in the paraventricular nucleus by scavenging nitric oxide that is released out of the neurons in which it is produced, but not that acting inside neurons. Indeed, the latter cannot be scavenged by hemoglobin, as the protein does not cross neuronal membranes because of its high molecular weight.

The involvement of endogenous nitric oxide at the paraventricular level in the control of penile erection in physiological contexts has been confirmed recently by microdialysis studies in male rats showing noncontact erections in the presence of an inaccessible receptive female and during copulation. Indeed, nitric oxide production measured as the concentration of nitrite and nitrate ions, the main metabolites of newly synthesized nitric oxide in biological tissues ([Ignarro, 1990](#)), increased in the extracellular dialysate obtained from the paraventricular nucleus of sexually potent male rats showing noncontact erections ([Melis et al., 1998](#)). Such increase was also found when copulation by the male with the receptive female was allowed ([Melis et al., 1998](#)). In both circumstances the increase in nitric oxide production was reduced by nitric oxide-synthase inhibitors given into the paraventricular nucleus, at doses that markedly reduced noncontact erections and impaired copulation ([Melis et al., 1998](#)). These findings support the hypothesis that nitric oxide activity is increased in the paraventricular nucleus during penile erection occurring in physiological sexual contexts. Further support for a role of paraventricular nitric oxide in the control of penile erection is also provided by immunocytochemical studies showing that nitric oxide-synthase content in the paraventricular nucleus of male rats selected for a low level of sexual activity with receptive females was only half that found in the paraventricular nucleus of male rats selected for a normal level of sexual activity ([Benelli et al., 1995](#)). This difference was not found in other brain areas involved in the control of sexual behavior, such as the medial amygdala and the bed nucleus of the stria terminalis ([Benelli et al., 1995](#)).

### 3.4. Oxytocin

That oxytocin induces penile erection in male rats was first reported in 1985 ([Argiolas et al., 1985](#)), but see also ([Argiolas et al., 1986](#)). In the meantime oxytocin was also found able to facilitate female and male sexual behaviour in rats ([Arletti and Bertolini, 1985](#); [Arletti et al., 1985](#); [Caldwell et al., 1986](#); [Gorzalka and Lester, 1987](#)). The sexual effects of oxytocin were further confirmed by experimental data in other laboratory animals, and are likely to occur also in humans, as plasma oxytocin concentration increases during sexual activity (for a review see, [Argiolas and Gessa, 1991](#); [Argiolas, 1999](#); and references therein). The paraventricular nucleus of the hypothalamus was soon identified as one of the most sensitive brain areas for the facilitatory effect of the peptide

on penile erection followed by the hippocampus (Melis et al., 1986). As oxytocin is effective at doses as low as 3 ng (e.g., 3 pmol) when injected in the paraventricular nucleus, the neuropeptide remains one of the most potent substances that induce penile erection discovered so far. Several lines of experimental evidence suggest that oxytocin given into the paraventricular nucleus induces penile erection by activating its own neurons projecting to extra-hypothalamic brain areas. Accordingly, the proerectile effect of the neuropeptide is abolished almost completely by bilateral electrolytic lesions of the paraventricular nucleus, which eliminate all central extra-hypothalamic oxytocinergic neurons originating in the paraventricular nucleus (Hawthorn et al., 1985), including those controlling penile erection, but not of surrounding regions (Argiolas et al., 1987a). Structure-activity relationship studies have also shown that oxytocin-induced penile erection is mediated by oxytocin uterine-type receptors (Argiolas et al., 1989b). The stimulation of these receptors, located on the cell bodies of oxytocinergic neurons, activates these neurons to release oxytocin in extra-hypothalamic brain areas, inducing in turn penile erection. Accordingly, oxytocin-induced penile erection is prevented by oxytocin receptor antagonists given not only into the paraventricular nucleus but also into the lateral ventricles, with a potency that is parallel to that of these compounds in blocking oxytocin receptors in the uterus (Argiolas et al., 1987b; Melis et al., 1997b). The mechanism through which the stimulation of oxytocinergic receptors activates paraventricular oxytocinergic neurons mediating penile erection is likely to be mediated by an increased  $\text{Ca}^{2+}$  influx as found in the uterus and mammary gland (see Argiolas and Gessa, 1991; Argiolas and Melis, 1995, 2004; Argiolas, 1999; Melis and Argiolas, 1993). This causes the activation of nitric oxide-synthase, increasing nitric oxide production. Nitric oxide in turn activates oxytocinergic neurons, which release oxytocin in extra-hypothalamic brain areas, as reported above for dopamine and excitatory amino acid receptors. In line with this hypothesis, oxytocin injected into the paraventricular nucleus at a dose that induces penile erection increases nitric oxide production in the hypothalamic nucleus (Melis et al., 1997b), and this effect is prevented either by the blockade of N-type voltage-dependent  $\text{Ca}^{2+}$  channels (Succu et al., 1998) or by nitric oxide-synthase inhibitors (Melis et al., 1997b), both given into the nucleus at doses that dramatically reduce penile erection.

The role of oxytocin and of paraventricular oxytocinergic neurons in the control of penile erection and sexual behaviour is also supported by many other studies. Accordingly, immunocytochemical studies have shown that oxytocin messenger RNA content in the paraventricular nucleus of male rats selected for a low level of sexual activity with receptive females is significantly lower than that found in the paraventricular nucleus of male rats selected for a normal level of sexual activity with receptive females (Arletti et al., 1997). This finding resembles the

lower content of nitric oxide synthase messenger RNA found in the paraventricular nucleus of male rats with low sexual activity when compared to male rats with normal sexual activity (Benelli et al., 1995). Yet, the blockade of central oxytocinergic receptors by nanogram amounts of oxytocin antagonists practically abolishes noncontact erections seen in sexually potent male rats put in the presence of an inaccessible receptive female (Melis et al., 1998, 1999a), male rat copulatory behaviour (Argiolas et al., 1988; Melis et al., 1998) and the facilitatory effect of dopamine agonists on male rat sexual behaviour (Argiolas et al., 1989a). Interestingly, sexual interaction increases Fos, the gene product of the immediate early gene *c-fos*, in paraventricular oxytocinergic neurons projecting to the spinal cord, which are involved in the control of penile erection (see Witt and Insel, 1994; Carter et al., 1997; and references therein). In contrast to the above findings, which support a facilitative role of oxytocin in erectile function and sexual behaviour, the neuropeptide is found able to reduce sexual activity in the prairie vole (see Carter, 1992; and references therein). Finally, oxytocinergic neurons mediate the proerectile effect of dopamine agonists, excitatory amino acids, nitric oxide donors and other substances such as hexarelin analogue peptides and VGF-derived peptides, as described in the specific sections dedicated to these compounds.

### 3.5. Hexarelin analogue peptides

Hexarelin analogue peptides derive from hexarelin, a peptide initially characterized for its ability to release growth hormone (GH) in laboratory animals and in humans with potency similar to that of natural GH-RH (Deghenghi, 1996; Deghenghi et al., 1994; Muller et al., 1999). Recently, a few of these peptides were found able to induce penile erection when injected into the paraventricular nucleus of the hypothalamus and, to a lesser extent, when given systemically (Melis et al., 2000c,d, 2001a) (Table 3). Some hexarelin analogue peptides injected into the paraventricular nucleus were found to have potency in inducing penile erection comparable on a molar basis to that of dopamine agonists, oxytocin and NMDA (Melis et al., 2000d). The available experimental evidence suggests that hexarelin analogues induce penile erection by activating paraventricular oxytocinergic neurons projecting to extra-hypothalamic brain areas. Indeed, their proerectile effect is reduced by the oxytocin antagonist  $\text{d}(\text{CH}_2)_5\text{Tyr}(\text{Me})^2\text{-Orn}^8\text{-vasotocin}$  given into the lateral ventricles but not into the paraventricular nucleus (Melis et al., 2001a). Structure-activity relationship studies suggest that the peptides with proerectile activity induce penile erection by stimulating specific receptors, other than those previously characterized that mediate growth hormone release and feeding behaviour (Melis et al., 2000d), and that are probably located on the cell bodies of oxytocinergic neurons mediating penile erection (Melis et al., 2001a). Apparently the activation of these receptors induces penile erection by increasing  $\text{Ca}^{2+}$

Table 3  
Structure of hexarelin analogue peptides

Hexarelin analogues	Amino acid sequence	Erectile activity
Hexarelin	His-D-Trp(2-Me)-Ala-Trp-D-Phe-LysNH <sub>2</sub>	Inactive
EP 40904	Thr-D-Trp(2-Me)-Ala-Trp-D-Phe-LysNH <sub>2</sub>	Inactive
EP 40737	D-Thr-D-Trp(2-Me)-Ala-Trp-D-Phe-LysNH <sub>2</sub>	Inactive
EP 50885	GAB-D-Trp(2-Me)-D-βNal-Phe-LysNH <sub>2</sub>	Active
EP 90101	GAB-D-Trp(2-Me)-D-βNal-Phe-ArgNH <sub>2</sub>	Active
EP 51322	GAB-D-Trp(2-Me)-D-βNal-NH <sub>2</sub>	Inactive
EP 60761	GAB-D-Trp(2-Me)-D-Trp(2-Me)-D-Trp(2-Me)-LysNH <sub>2</sub>	Active
EP 70555	GAB-D-Trp(2-Me)-D-Trp(2-Me)-D-Trp(2-Me)-Arg(NO <sub>2</sub> )NH <sub>2</sub>	Inactive
EP 51216	GAB-D-Trp(2-Me)-D-Trp(2-Me)-L-Trp(2-Me)-LysNH <sub>2</sub>	Inactive
EP 80661	GAB-D-Trp(2-Me)-D-Trp(2-Me)-LysNH <sub>2</sub>	Active
EP 91072	GAB-D-Trp-D-Trp-LysNH <sub>2</sub>	Active
EP 91073	AIB-D-Trp(2-Me)-D-Trp(2-Me)-LysNH <sub>2</sub>	Inactive <sup>a</sup>
EP 51389	AIB-D-Trp(2-Me)-D-Trp(2-Me)-NH <sub>2</sub>	Inactive

Abbreviations: GAB,  $\gamma$ -amino-butyryl; AIB, amino-isobutyryl;  $\beta$ Nal,  $\beta$ -(2-naphthyl)-alanine.

<sup>a</sup> This peptide acts as an antagonist of hexarelin analogue peptide receptors mediating penile erection (see Melis et al., 2001a).

influx on the cell bodies of oxytocinergic neurons, which causes the activation of nitric oxide-synthase, as reported for dopamine agonists, oxytocin and NMDA. Nitric oxide in turn activates oxytocinergic neurons as mentioned above (see Sections 3.3 and 3.4). Accordingly, hexarelin analogue peptide-induced penile erection occurs concomitant with an increased nitric oxide production in the paraventricular nucleus and is prevented by the inhibition of paraventricular nitric oxide-synthase (Melis et al., 2000c, 2001c) and by the blockade of N-type voltage dependent Ca<sup>2+</sup> channels by  $\omega$ -conotoxin (Melis et al., 2000c), as well as by oxytocin receptor antagonists given into the lateral ventricles but not in the paraventricular nucleus (Melis et al., 2000c,d). The recent discovery of a hexarelin analogue peptide devoid of proerectile activity that prevents hexarelin analogue peptide-induced penile erection, but not that induced by apomorphine, oxytocin or NMDA, together with the data summarized above, seems to confirm the existence of a specific receptor for these hexarelin analogue peptides in the paraventricular nucleus, the stimulation of which induces penile erection (Melis et al., 2001a). In this regard, it is noteworthy that an endogenous acylated peptide that acts on the same receptors activated by hexarelin, its analogues and other growth hormone secretagogues, to release growth hormone and to increase feeding behaviour, e.g., ghrelin, has been recently isolated and characterized from the stomachs of rodents and of humans (Kojima et al., 1999). Ghrelin has also been identified in the hypothalamus and in other brain areas (Hosoda et al., 2000). However, ghrelin injected into the paraventricular nucleus induces feeding, but not penile erection (Melis et al., 2002) (Fig. 4), a finding in line with the existence of a specific receptor of hexarelin analogue peptides, the activation of which leads to penile erection and that is different from those that induce feeding or growth hormone release. It is tempting to speculate that an endogenous hexarelin analogue peptide-related substance that acts on the same receptors activated by proerectile hexarelin analogue peptides exists in the paraventricular nucleus that controls oxytocinergic neurons mediating

penile erection. However, the possibility that proerectile hexarelin peptides interfere with some other endogenous substance present in the paraventricular nucleus involved in the control of penile erection and as yet unidentified cannot be completely ruled out.

### 3.6. Cannabinoid CB1 receptor antagonists

The ability of the cannabinoid CB1 receptor antagonist SR 141716A [*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide] to induce penile erection when injected into the paraventricular nucleus of male rats was discovered only recently (Melis et al., 2004a). In contrast to SR 141716A, the cannabinoid CB1 receptor agonists WIN 55,212-2 [4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6H-pyrrolo[3,2,1-*I*,*j*]quinolin-6-one] and CP 55,940 ([1 $\alpha$ ,2 $\beta$ -(*R*)-5 $\alpha$ ]-5-(1,1-dimethylheptyl)-2-[5-hydroxy-2-(3-hydroxy-propyl)cyclohexyl]phenol) were found ineffective. Nevertheless, both compounds reduced the enhancing effect of SR 141716A on penile erection when given into the paraventricular nucleus before SR 141716A (Melis et al., 2004a). The proerectile effect of SR 141716A

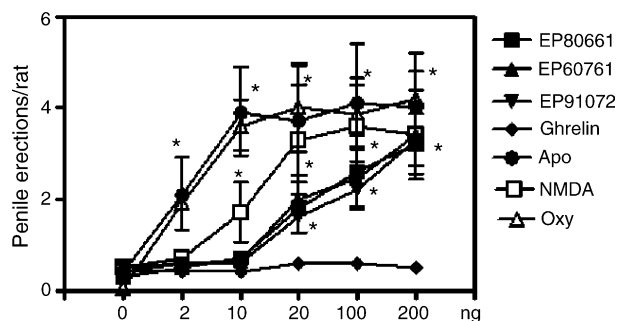


Fig. 4. Penile erection induced by hexarelin analogue peptides injected into the PVN: comparison with ghrelin, apomorphine, NMDA and oxytocin. The compounds were dissolved in saline and injected into the paraventricular nucleus in a volume of 0.3  $\mu$ l in 2 min. Values are means  $\pm$  S.E.M. of seven rats per group. \**P* < 0.01 with respect to control rats (one-way ANOVA).

was also reduced by the blockade of NMDA receptors and by nitric oxide synthase inhibition but not by the blockade of dopamine or oxytocin receptors in the paraventricular nucleus, while it was prevented by the blockade of central oxytocin receptors by oxytocin receptor antagonists given into the lateral ventricles (Melis et al., 2004a). This has led to the suggestion that the blockade of cannabinoid CB1 receptors present in the paraventricular nucleus at a site(s) yet to be identified may influence erectile function and sexual activity by increasing the activity of excitatory amino acid neurotransmitters (possibly glutamic acid), which in turn activate paraventricular oxytocinergic neurons mediating erectile function (Melis et al., 2004a). In this regard, it is pertinent to recall that cannabinoid receptors of the CB1 subtype are present in the paraventricular nucleus (Herkenham et al., 1991). These receptors are often found to be coupled in different neural tissues to the inhibition of voltage-dependent  $Ca^{2+}$  channels and usually cause the inhibition of neurotransmitter release from neural synapses bearing these receptors (see Mackie and Hille, 1992; Pan et al., 1998; Twitchell et al., 1997). Interestingly, among the numerous effects induced by the systemic administration of endogenous and exogenous cannabinoids (motor disturbances, hypothermia, analgesia and endocrine effects exerted mainly through the hypothalamus, including the paraventricular nucleus) (see Chaperon and Thiebot, 1999; and references therein) are also included inhibitory effects on penile erection and male sexual behaviour (see Ferrari et al., 2000; Shrenker and Bartke, 1985; and references therein), and SR 141716A has been reported able to facilitate the proerectile effect of apomorphine (da Silva et al., 2003). Although further studies are necessary to identify the exact mechanism by means of which the blockade of cannabinoid CB1 receptors influence erectile function and sexual activity, the results summarized above suggest that the paraventricular nucleus may be one of the brain sites where cannabinoid CB1 receptors may act to influence sexual function possibly by modulating oxytocinergic neurotransmission. Further support to this hypothesis comes from microdialysis experiments showing that the proerectile effect of SR-141716A injected into the paraventricular nucleus occurs concomitantly with an increase in nitric oxide production, and that this effect is reduced by paraventricular

nitric oxide synthase inhibition, as found for other agents that facilitate the erectile response when injected into the paraventricular nucleus (Melis and Argiolas, in preparation).

### 3.7. Pro-VGF-derived peptides

The ability of a few pro-VGF-derived peptides to induce penile erection when injected into the paraventricular nucleus of male rats has been discovered only recently, and it is still under investigation. These peptides derive from the proteolytic cleavage of VGF, the primary translation product of the *vGF* gene, originally identified because its mRNA was selectively induced by nerve growth factor (NGF) in PC12 cells (Levi et al., 1985, 2004) (see Table 4 for amino acid sequence). So far the effect of five peptides derived from the C terminal portion of rat pro-VGF, VGF<sub>577–617</sub> or HHPD 41, VGF<sub>588–617</sub> or AQEE 30, VGF<sub>599–617</sub>, VGF<sub>556–576</sub> or TLQP 21 and VGF<sub>588–597</sub> or LQEQ 19 on penile erection was studied in some detail after injection into the hypothalamic paraventricular nucleus. VGF<sub>577–617</sub>, VGF<sub>588–617</sub>, VGF<sub>599–617</sub> and, to a lower extent, VGF<sub>588–597</sub> were found capable of inducing penile erection in a dose dependent manner, while VGF<sub>556–576</sub> was found ineffective (Succu et al., 2004). Hence, the proerectile amino acid sequence seems to be localized in the last 30 amino acids of the pro-VGF C-terminus. As VGF<sub>588–617</sub>-induced penile erection was reduced by  $N^G$ -nitro-L-arginine methylester (L-NAME), which inhibits nitric oxide synthase, and by  $d(CH_2)_5Tyr(Me)-Orn^8$ -vasotocin, a potent oxytocin receptor antagonist, when given into the lateral ventricles, but not when injected into the paraventricular nucleus (Succu et al., 2004), it is likely that also pro-VGF-derived peptides facilitate erectile function by increasing oxytocinergic neurotransmission (Fig. 5). In line with this hypothesis, immunocytochemistry studies with antibodies raised against the C terminal nonapeptide sequence of pro-VGF (VGF<sub>609–617</sub>) have revealed numerous neuronal fibres and terminals within the paraventricular nucleus, including its parvocellular components, and perhaps more important, many VGF-immunostained neuronal terminals impinging onto parvocellular oxytocinergic neurons (Succu et al., 2004). As the tested pro-VGF-derived peptides have been isolated and

Table 4  
Amino acid sequence of pro-VGF peptides

VGF peptide	Amino acid sequence	Erectile activity
VGF <sub>556–576</sub> (TLQP 21)	TLQPPASSRRRHFFHALPPAR	Inactive
VGF <sub>577–617</sub> (HHPD 41)	HHPDLEAQARRAQEEADAERRLQEQEELNYIEHVLLHRP	Active
VGF <sub>588–617</sub> (AQEE 30)	AQEEADAERRLQEQEELNYIEHVLLHRP	Active
VGF <sub>599–617</sub> (LQEQ 19)	LQEQEELNYIEHVLLHRP	Active

Amino acids are shown with the one-letter amino acid code.

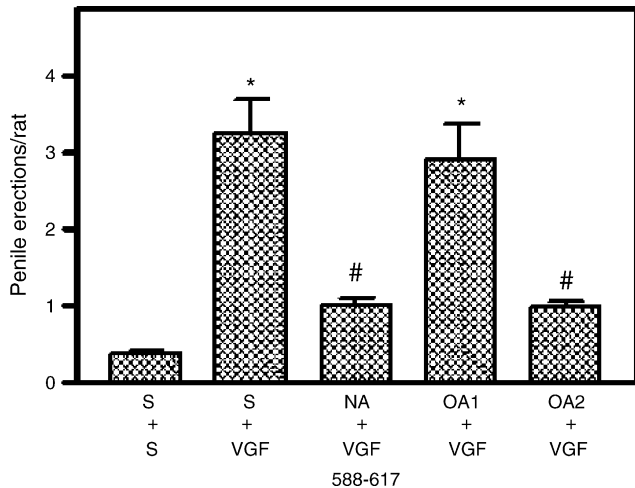


Fig. 5. Effect of  $N^G$ -nitro-L-arginine methylester (L-NAME) and  $d(\text{CH}_2)_5$ -Tyr(Me)-Orn<sup>8</sup>-vasotocin on VGF<sub>588-617</sub>(AQEE 30)-induced penile erection. VGF<sub>588-617</sub> (1  $\mu\text{g}$ ) was injected into the paraventricular nucleus in a volume of 0.3  $\mu\text{l}$  in 2 min of saline. L-NAME (25  $\mu\text{g}$ )(NA),  $d(\text{CH}_2)_5$ -Tyr(Me)-Orn<sup>8</sup>-vasotocin (1  $\mu\text{g}$ )(OA1) were injected into the paraventricular nucleus in a volume of 0.3  $\mu\text{l}$  in 2 min 15 min before the VGF peptide.  $d(\text{CH}_2)_5$ -Tyr(Me)-Orn<sup>8</sup>-vasotocin (1  $\mu\text{g}$ )(OA2) was also injected into the lateral ventricles 15 min before the VGF peptide. Values are means  $\pm$  S.E.M. of seven rats per group. \* $P < 0.01$  with respect to saline-treated rats; # $P < 0.01$  with respect to the corresponding group treated with the VGF peptide alone (one-way ANOVA).

characterized from the rat brain by the combined use of high-resolution separation techniques, matrix assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry and manual Edman degradation (Trani et al., 2002), the available data suggest that, within this nucleus, these or closely related pro-VGF-derived peptides may be released under physiological circumstances to influence sexual function by activating paraventricular oxytocinergic neurons mediating penile erection. This is also supported by microdialysis studies showing that the proerectile effect of VGF peptides occurs concomitantly with an increase in paraventricular nitric oxide production, an increase which is reduced by nitric oxide synthase inhibition, as found with other compounds that induce penile erection when injected into the paraventricular nucleus (Succu et al., 2005). However, even if this hypothesis will prove to be true, further experiments are necessary to clarify the exact mechanism by means of which these peptides activate paraventricular oxytocinergic neurons. Nevertheless, it is noteworthy that the absence of pro-VGF protein and its derived peptides, as it occurs in VGF-knockout mice, resulted in dramatically impaired sexual behaviour, sexual maturation and fertility (Hahm et al., 1999; Salton et al., 2000).

### 3.8. ACTH-MSH-related peptides

ACTH-MSH-related peptides are another group of neuropeptides that induce penile erection by acting in the

hypothalamus (for a review see, Argiolas, 1999; Schioth, 2001; Martin and McIntyre, 2004; and references therein). Apparently the proerectile effect of these neuropeptides is mediated by the stimulation of hypothalamic melanocortin MC receptors of the MC<sub>3</sub> and MC<sub>4</sub> subtype (Vergoni et al., 1998; Argiolas et al., 2000; Martin et al., 2002). However very little evidence is available that these neuropeptides activate paraventricular oxytocinergic neurons to induce penile erection. Indeed, these neuropeptides induce penile erection when injected not only in the paraventricular nucleus but also in all the periventricular area surrounding the third ventricle (Argiolas et al., 2000). Moreover, early studies have shown that their proerectile effect is scarcely influenced by bilateral electrolytic lesions of the paraventricular nucleus (Argiolas et al., 1987a), or by classical oxytocin receptor antagonists (Argiolas et al., 1987b). The latter finding was recently confirmed by studies showing that the increase in intracavernosal pressure induced by  $\alpha$ -MSH is not antagonized by a classical oxytocin receptor antagonist in contrast to that induced by oxytocin (Mizusawa et al., 2002). In contrast to the above studies, the proerectile effect of THIQ ( $N$ -[ $(3R)$ -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), a nonpeptide putative MC<sub>4</sub> receptor agonist found capable of inducing penile erection when injected systemically in male rats, has been recently reported to be partially reduced by L-368899, a nonpeptide oxytocin receptor antagonist selected for its ability to prevent oxytocin-induced contraction in uterine membranes, but never tested for its ability to reduce oxytocin-induced penile erection (Martin et al., 2002). The discrepancy between these studies is probably apparent only because the reduction by L-368899 of THIQ-induced penile erection reported in this study is only 20% (Martin et al., 2002). From the available data, it seems then that ACTH-MSH peptides and nonpeptide melanocortin MC agonists induce penile erection by acting on MC<sub>3</sub> and/or MC<sub>4</sub> receptors in the periventricular hypothalamic region of the third ventricle, which includes also the paraventricular nucleus, but the role of oxytocin, if any, in the proerectile effect of these peptides is very modest.

### 4. Neurotransmitters and/or neuropeptides that inhibit paraventricular oxytocinergic neurons impair penile erection

The endogenous neurotransmitters and/or neuropeptides that inhibit oxytocinergic neurons at the paraventricular level and reduce penile erection identified so far are  $\gamma$ -amino-butyric acid (GABA), the main inhibitory neurotransmitter present in the brain, and opioid peptides. Both neuromediators apparently act by stimulating specific receptors located on the cell bodies of oxytocinergic neurons, although indirect effects cannot be ruled out. This,

in turn, causes a decrease in the release of oxytocin in extra-hypothalamic sites distant from the paraventricular nucleus, e.g., the hippocampus, medulla oblongata and spinal cord. Exogenous substances that mimic the action of GABA or of opioid peptides (opiates), acting as agonists of the receptors for these compounds, often reduce drug-induced penile erection and noncontact erections when injected into the paraventricular nucleus.

#### 4.1. GABA

Although GABA is the main inhibitory neurotransmitter present in the brain, the study of its role in the control of penile erection and sexual behaviour has been always complicated by the often dramatic effects of GABAergic drugs on motor behaviour and vigilance (Hobbs et al., 1996). We reported recently that muscimol, a potent GABA<sub>A</sub> receptor agonist, injected into the paraventricular nucleus of male rats at doses that do not induce any gross behavioural change, inhibits penile erection induced by dopamine agonists, NMDA and oxytocin (Melis et al., 2000b), hexarelin analogue peptides (Succu et al., 2003), and pro-VGF-derived peptides (Succu et al., 2004), as well as noncontact penile erections (Melis et al., 2001b; Melis and Argiolas, 2002). The inhibitory effects of muscimol on drug-induced penile erection and on noncontact erections are elicited by the stimulation of GABA<sub>A</sub> receptors, since they are prevented by bicuculline, a classic GABA<sub>A</sub> receptor antagonist, given into the paraventricular nucleus, and are not observed after the injection of baclofen, a GABA<sub>B</sub> receptor agonist into the paraventricular nucleus (Melis et al., 2000b, 2001b; Melis and Argiolas, 2002; Succu et al., 2003). Since GABAergic nerve terminals impinge on magnocellular and parvocellular oxytocinergic cell bodies in the paraventricular nucleus of the hypothalamus (Boubada et al., 1996; Roland and Sawchenko, 1983; Jourdain et al., 1999), GABA<sub>A</sub> receptors whose stimulation inhibits penile erection are probably located on the cell bodies of oxytocinergic neurons mediating this sexual response. As to the mechanism by means of which the stimulation of GABA<sub>A</sub> receptors in the paraventricular nucleus prevents the activation of oxytocinergic neurons, and hence penile erection, it is pertinent to recall that the inhibitory effect of muscimol on penile erection occurs concomitantly with a reduction in the increase of nitric oxide production that occurs in this hypothalamic nucleus during drug- and oxytocin-induced penile erection and noncontact erections, and that this effect is prevented by bicuculline, which blocks GABA<sub>A</sub> receptors, given in the paraventricular nucleus before muscimol (Melis and Argiolas, 2002; Succu et al., 2003). All together, these findings suggest that the stimulation of GABA<sub>A</sub> receptors inhibits drug-induced and noncontact penile erection by decreasing the activity of nitric oxide-synthase in oxytocinergic neurons mediating penile erection. This causes, in turn, a decrease in the release of oxytocin in extra-hypothalamic brain areas involved in

the control of penile erection, impairing erectile function. The above explanation is based mainly on the assumption that GABA<sub>A</sub> receptors mediating the inhibition of penile erection are located mainly on the cell bodies of oxytocinergic neurons. However, it is impossible at present to rule out indirect effects of GABA<sub>A</sub> receptor agonists, that is, effects mediated by an action of these compounds on the activity of neurotransmitters and/or neuropeptides, which influence directly penile erection at the paraventricular level. Further studies are necessary to verify such possibility.

#### 4.2. Opioid peptides, morphine and other opiates

Morphine and other opiates exert a marked inhibitory effect on penile erection and male sexual behaviour, not only in laboratory animals but also in humans (for a review see Pfaus and Gorzalka, 1987; Argiolas, 1999). As these drugs are agonists of the various opioid receptor subtypes ( $\mu$ ,  $\kappa$  and  $\delta$ ) of endogenous opioid peptides (e.g., endorphins, enkephalins and dynorphins) (Pfaus and Everitt, 1995), it is generally accepted that opioid peptides exert an inhibitory role in the control of sexual behaviour. Accordingly, opiate addicts often complain of impotence and of a decrease in sexual libido, and penile erection is a common sign of opiate abstinence (Cushman, 1972) (see also Argiolas, 1999; and references therein). In line with an inhibitory effect of opioid peptides and opiates on erectile function, morphine was reported to prevent penile erection induced by apomorphine, by oxytocin and by NMDA when injected into the paraventricular nucleus (Melis et al., 1992b, 1997c,d). As the inhibitory effect of morphine on penile erection induced by the above compounds was prevented by naloxone, a classical opioid receptor antagonist, and as U-69,593, an opioid agonist 500 times more potent than morphine on the  $\kappa$  receptor subtype, was unable to prevent the facilitatory effect of apomorphine, NMDA and oxytocin on penile erection when given into the paraventricular nucleus (Melis et al., 1992b, 1997c,d), it was suggested that morphine inhibits penile erection by stimulating opioid receptors of the  $\mu$  subtype (see Argiolas, 1999; and references therein). However, a role of the other opioid receptor subtypes cannot be completely ruled out from the available data.

As the cell bodies of oxytocinergic neurons in the paraventricular nucleus are rich in opioid receptors mainly of the  $\mu$  subtype (Muhlethaler et al., 1980), one of the mechanisms by means of which morphine injected into the paraventricular nucleus may reduce penile erection is the inhibition of oxytocinergic neurons mediating this sexual response (Melis et al., 1992b). The mechanism by means of which the stimulation of opioid receptors causes the inhibition of oxytocinergic neurons is unknown at present. However, since microdialysis studies show that the prevention by morphine of penile erection induced by dopamine agonists, oxytocin and NMDA occurs concomitantly to a reduction of the increase in nitric oxide production seen in the paraventricular nucleus after injection

of a dose of the above substances that induces penile erection (Melis et al., 1997c,d), it is likely that the stimulation of opioid receptors by the opiate reduces nitric oxide-synthase activity. This causes in turn a reduction in nitric oxide production and in the activation of oxytocinergic neurons. In line with this hypothesis, morphine injected into the paraventricular nucleus reduces penile erection induced by not only dopamine agonists, oxytocin and NMDA, but also by hexarelin analogue peptides (Succu et al., 2003) and by pro-VGF-derived peptides (Succu et al., 2004). Morphine injected into the paraventricular nucleus reduces also noncontact penile erections that occur in male rats in the presence of an inaccessible receptive female and during copulation (Melis et al., 1999b; Argiolas, 1999). As found with drug- and peptide-induced penile erection, these inhibitory effects of morphine also occur parallel to a decrease in the increased nitric oxide production found in these physiological contexts (Melis et al., 1998, 1999b). Further support for an inhibitory role of endogenous opioid peptides and opiates at the paraventricular level on penile erection and sexual behavior is provided by studies showing that male rats with a low level of sexual activity are characterized by levels of opioid peptide messenger RNAs in the paraventricular nucleus higher than those found in that of male rats with a normal level of sexual activity, in addition to lower levels of oxytocin and nitric oxide-synthase messenger RNAs (Benelli et al., 1995; Arletti et al., 1997) (see also Sections 3.3 and 3.4).

## 5. Conclusions

The results of the studies reviewed above support a physiological role of the paraventricular nucleus of the hypothalamus in the control of erectile function mainly in the male rat. In particular, these studies provide evidence that a group of oxytocinergic neurons originating in this hypothalamic nucleus and projecting to extra-hypothalamic brain areas and to the spinal cord, including the spinal nuclei from which peripheral and autonomic nervous pathways originate to reach the penile erectile tissues, is involved in the control of penile erection (see Figs. 1 and 2). When activated, these neurons release oxytocin and induce penile erection, whilst when inhibited, penile erection is reduced or impaired. Numerous neurotransmitters and/or neuropeptides present in the paraventricular nucleus are capable of activating oxytocinergic neurons and inducing penile erection. These include dopamine, excitatory amino acids, nitric oxide and oxytocin itself. Hexarelin analogue peptides and pro-VGF-derived peptides have recently been added to this list; however, whether this indicates the existence of other endogenous substances capable of activating oxytocinergic neurons at the paraventricular level and thus influencing erectile function has still to be verified. In this regard, it is pertinent to recall that while pro-VGF-derived peptides have been identified in brain (Trani et al., 2002),

this has yet to be proved for an endogenous hexarelin analogue compound, since ghrelin, an endogenous ligand for growth hormone secretagogues, does not induce penile erection when injected into the paraventricular nucleus (Melis et al., 2002). A possible modulatory role of paraventricular endocannabinoids in the control of erectile function has also been discovered, as the blockade of cannabinoid CB1 receptors by SR 141716A in the paraventricular nucleus induces penile erection (Melis et al., 2004a), although further experiments are necessary to clarify in detail such a role. Opioid peptides and GABA are the only endogenous substances identified to date that inhibit oxytocinergic neurons and penile erection by acting at the paraventricular level. Apparently, opioid-like compounds act mainly through opioid receptors of the  $\mu$  subtype, and GABA agonists act mainly through GABA<sub>A</sub> receptors. Interestingly, the activation/inhibition of oxytocinergic neurons is apparently secondary to the activation/inhibition of paraventricular nitric oxide synthase, the enzyme that produces nitric oxide from L-arginine. This makes this recently discovered neuromodulator another important endogenous mediator that controls erectile function at the paraventricular level, in addition to its primary role as a physiological mediator of the relaxation of the corpora cavernosa at the local level (Burnett et al., 1992; Rajfer et al., 1992).

Despite the important physiological role suggested for oxytocin and its central neurons in the control of erectile function and sexual activity in rodents and other mammals, it is important to recall that oxytocin gene ablation has been found to produce oxytocin knock out mice that mate and copulate normally, as if oxytocin was not necessary for mating and copulation (Nishimori et al., 1996; Young et al., 1996). Also the homozygous female oxytocin knock out mice show normal mating and parturition, although with a marked impairment of milk let-down (Nishimori et al., 1996; Young et al., 1996). However, the ablation of the gene encoding neuronal nitric oxide-synthase also produces nitric oxide-synthase knock out mice that mate and copulate normally (Huang et al., 1993). This occurs although nitric oxide-synthase produces nitric oxide, which is one of the main physiological mediators of penile erection at the penile level (Burnett et al., 1992; Rajfer et al., 1992) and at the central level, in the paraventricular nucleus of the hypothalamus (Argiolas, 1994; Melis et al., 1998; Arletti et al., 1997) (see Section 3.3). It is likely that these findings probably reflect the redundancy of the systems involved in the control of reproductive physiology at central and peripheral levels. Such redundancy certainly has an evolutionary origin, since it guarantees the passage of genes to the next generation for the survival of the species. Therefore, the fact that ablation of the genes encoding oxytocin or nitric oxide-synthase does not alter the reproductive function and behaviour may simply mean that oxytocin and nitric oxide are only two of the mediators working in the systems controlling this complex function,

rather than suggesting that there is no role for oxytocin or nitric oxide in the control of sexual behaviour (see also Argiolas, 1999). In line with this possibility, recent studies suggest that vasopressin may partially compensate for the lack of effect of oxytocin in these oxytocin knock out mice. Indeed, vasopressin was found able to stimulate central oxytocinergic receptors more effectively in oxytocin knock out mice when compared to oxytocin wild type mice (Ragnauth et al., 2004). Similarly, vasopressin neurons are activated in hypothalamic nuclei during parturition, suggesting that, even in wild type female mice, vasopressin neurons may contribute to parturition (Douglas et al., 2002). Similar considerations may be made for neuronal nitric oxide knock out mice, as recent studies have shown that these original knock out mice contain residual neuronal nitric oxide synthase due to expression of  $\beta$ - and  $\gamma$ -neuronal nitric oxide splice variant forms (Gyurko et al., 2002). Furthermore, neuronal nitric oxide synthase knock out male mice that lack exon 6, the heme binding domain of neuronal nitric oxide synthase, are unable to copulate, despite normal sperm counts (Gyurko et al., 2002). Hence, a complete elimination of neuronal nitric oxide synthase really interferes with erectile function and sexual behavior.

In conclusion, a group of paraventricular oxytocinergic neurons projecting to extra-hypothalamic brain areas and to the spinal cord is involved in the control of erectile function in male rats. These neurons may be activated or inhibited by numerous endogenous neurotransmitters and/or neuropeptides. As these endogenous substances induce facilitatory or inhibitory effects on erectile function by acting on specific receptors, this renders paraventricular oxytocinergic neurons an interesting target for the physiological and pharmacological central control of penile erection, and makes the paraventricular nucleus of the hypothalamus an important area for the central control of erectile function and sexual activity. Although this conclusion derives from studies performed in rats, mainly by manipulating neural activity in this hypothalamic nucleus with drugs injected directly into the nucleus, making it difficult to distinguish physiological from pharmacological effects of these compounds, these findings have great relevance in a human perspective for the treatment of erectile dysfunction for several reasons. First, oxytocinergic neurons originating in the paraventricular nucleus of the hypothalamus and projecting to extrahypothalamic brain areas including the spinal cord are also present in humans already at the time of perinatal life, with an organization resembling that found in rats (see Sofroniew, 1983; Mai et al., 1997). Second, oxytocinergic neurotransmission is increased during sexual activity not only in male rats (see Section 3.4) but also in humans, as shown by the increase in plasma oxytocin concentration in men engaged in sexual activity, especially at ejaculation, and in women after manipulation of breast and/or genitalia, all stimuli that occur during sexual intercourse (Tindall, 1974; Murphy et al., 1987; Carmichael et al., 1987). Finally, the male rat is a well-recognized model

in the field of sexual behaviour and largely used in the field of penile physiology. Although rats do not compare completely penile anatomy, neuroanatomy and physiology with humans (see Sachs and Meisel, 1988; Hull et al., 2002, for details), these animals are a predictive model for human penile erection and sexual behaviour. Indeed, the rat model of penile physiology has largely contributed in the last 15 years to increasing our knowledge of the peripheral and central mechanisms controlling erectile function, and drugs that induce penile erection in rats usually do so also in man. Some of the drugs identified for their proerectile effect in rats have been very successful in the therapy of erectile dysfunction (e.g., orally active phosphodiesterase type V inhibitors, which are today the most popular treatment for erectile dysfunction), while others have not been so efficacious as expected (e.g., apomorphine, although this is due to collateral effects of this drug, as nausea and vomiting, and not to its inability to facilitate erectile function in man). The clinical success of phosphodiesterase inhibitors acting peripherally (in the corpora cavernosa) versus the modest effect of apomorphine acting centrally in treating erectile dysfunction undoubtedly supports the idea that, at the moment, the best way to treat such dysfunction is to target peripheral tissues rather than the brain (see Argiolas, 2005). However, it is to be hoped that this will not cause a reduction in research efforts aimed at identifying further strategies for the therapy of erectile dysfunction, as the kinds of studies reviewed above are fundamental for increasing our understanding of the central physiological mechanisms controlling erectile function and sexual behaviour in man.

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