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Activation of $GABA_A$ and opioid receptors reduce penile erection induced by hexarelin peptides

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Abstract

The effect of muscimol, a $GABA_A$ receptor agonist, and of morphine, an opioid receptor agonist, on penile erection induced by the hexarelin analogue peptide EP 80661 (GAB-D-Trp(2-Me)-D-Trp(2-Me)-$LysNH_2$) and on the increase in the concentration of NO_2^- and NO_3^- that occurs concomitantly in the dialysate obtained from the paraventricular nucleus (PVN) of the hypothalamus by intracerebral microdialysis, was studied in male rats. Muscimol (50, 100 and 200 ng) and morphine (0.1, 0.5, 1 and 5 μg) given into the PVN dose-dependently reduced penile erection induced by EP 80661 (1 μg) injected into the PVN. The reduction of penile erection was parallel to a decrease of the concomitant NO_2^- and NO_3^- increase that occurs in the paraventricular dialysate in these experimental conditions. Muscimol and morphine effects on EP 80661-induced penile erection and NO_2^- increase were prevented by the prior administration into the PVN of bicuculline (250 ng) and naloxone (5 μg), respectively. The present results show that the activation of $GABA_A$ receptors and of opioid receptors in the PVN reduces penile erection induced by hexarelin analogue peptides by reducing the increase in NO activity that occurs in this hypothalamic nucleus in these experimental conditions.

Keywords: GABA; Muscimol; Morphine; Naloxone; Hexarelin analogue peptides; Oxytocin; Nitric oxide (NO); Penile erection

1. Introduction

A few peptide analogues of hexarelin, a peptide characterised by its ability to release growth hormone (GH) in animals and man (Deghenghi, 1996; Deghenghi et al., 1994; Muller et al., 1999, and references therein), induce penile erection and increase intracavernosal pressure when injected into the paraventricular nucleus (PVN) of the hypothalamus or systemically in male rats (see Melis et al., 2000; Giuliano et al., 2001). Structure–activity comparisons revealed that the proerectile effect of these peptides was not related to their effect on GH release or eating behaviour (Melis et al., 2000). This led us to suggest that these hexarelin analogues induce penile erection by acting on specific receptors, which differ from those that mediate GH release or eating behaviour (Melis et al., 2000). Among the analogues tested, GAB-D-Trp(2-Me)-D-Trp(2-Me)-$LysNH_2$ (EP 80661) was active in inducing the sexual response at doses as low as 20 ng. The proerectile effect of these peptides was not antagonised by injection into the PVN of oxytocin, dopamine or *N*-methyl-D-aspartic acid (NMDA) receptor antagonists, but was reduced by ω-conotoxin-GVIA, a potent N-type Ca^{2+} channel blocker (McCleskey et al., 1987) given into the PVN, by N^G-nitro-L-arginine methylester (L-NAME), a potent competitive inhibitor of the Ca^{2+}/calmodulin-dependent enzyme nitric oxide (NO) synthase (Rees et al., 1990), given in the lateral ventricles (intracerebroventricularly) or into the PVN, and by $[d(CH_2)_5Tyr(Me)^2Orn^8]$vasotocin, a potent oxytocin receptor antagonist (Bankowski et al., 1980), given intracerebroventricularly but not into the PVN (see Melis et al., 2000, and references therein). Similar results are found when penile erection is induced by dopamine receptor agonists, oxytocin and NMDA (Argiolas and Melis, 1995; Melis et al., 1996, 1997a,b) and when penile erection occurs in physiological contexts (see Melis et al., 1998, 1999a,b, and references therein). Accordingly, we suggest that hexarelin analogues may activate oxytociner-

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gic neurons mediating penile erection by activating NO synthase in the PVN. In line with this hypothesis, penile erection induced by proerectile hexarelin analogues occur concomitantly to an increase in NO production in the PVN (Melis et al., 2001a,b) as measured by the increase of the concentrations of NO_2^- and NO_3^-, which represent the reaction products of newly formed NO with O_2 and a reliable although indirect indicator of NO production in vivo (see Melis et al., 1996, and references therein), in the dialysate collected from a vertical microdialysis probe implanted in the PVN. This increase in NO production by hexarelin analogue peptides is prevented by NO synthase inhibitors given into the PVN at doses that also reduce penile erection (Melis et al., 2001a,b).

The activation of $GABA_A$ receptors in the PVN by muscimol reduces penile erection (and yawning) induced by apomorphine, oxytocin and NMDA, and noncontact penile erections (see Melis and Argiolas, 2002). These inhibitory effects of muscimol on erectile activity are manifested concomitantly to a reduction in the increase of NO production occurring in the PVN during these sexual responses (Melis and Argiolas, 2002) These findings are in line with immunocytochemical and electrophysiological studies showing that paraventricular magnocellular and parvocellular oxytocinergic neurons are under inhibitory GABAergic control and suggest that GABA is also involved at the PVN level in the control of erectile function. Accordingly, GABA synapses impinge on the cell bodies of oxytocinergic neurons (Boubada et al., 1996; Jourdain et al., 1999; Roland and Sawchenko, 1993), and GABA agonists decrease oxytocinergic transmission in several circumstances (see Bisset et al., 1990; Voisin et al., 1995). Most of these inhibitory synapses belong to GABAergic neurons originating mainly in the perinuclear region that surrounds the PVN (Boubada et al., 1996; Roland and Sawchenko, 1993). A marked reduction of apomorphine-, oxytocin- and NMDA-induced penile erection and of noncontact erections, occurring concomitantly to a reduction in the increase in paraventricular NO production, is also found when morphine, an opioid receptor agonist, is injected into the PVN of male rats, as these inhibitory effects of morphine are prevented by the prior administration of naloxone, a classical opioid receptor antagonist (see Melis et al., 1999a, and references therein). Interestingly, morphine is also able to reduce hexarelin analogue peptide-induced penile erection (see Melis et al., 2001a,b, and references therein). To further characterize the role of GABA and of opioid receptors at the PVN level in the control of penile erection and the mechanism through which hexarelin analogue peptides induce penile erection by acting in the PVN, we studied the effect of muscimol and of morphine on penile erection induced by the proerectile hexarelin analogue EP 80661 and on the increase of paraventricular NO production that occurs concomitantly to the sexual response induced by this peptide.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (250–300 g) (Charles River, Como, Italy) were used in all the experiments. The animals were caged in groups of four to six at 24 °C, humidity 60%, lights on from 0700 to 1900 h, with water and standard laboratory food ad libitum. The experiments were performed between 0900 and 1300 h. All experiments were performed in accordance with the guidelines of the European Communities Directive of November 24, 1986 (86/609/EEC), and the Italian Legislation (D.P.R. 116/92).

2.2. Drugs and peptides

The hexarelin analogue peptide EP 80661, prepared by conventional solid-phase synthesis and purified by high-pressure liquid chromatography (HPLC), was a kind gift of Dr. Romano Deghenghi. Muscimol and bicuculline methiodide were purchased from Sigma (St. Louis, MO, USA), morphine-HCl and naloxone-HCl from Research Biochemical International (Natick, MA, USA). All other reagents were of the highest available purity.

2.3. Microinjections and microdialysis in the PVN

Microinjections and microdialysis were performed in the PVN of the same male rat by using microdialysis probes (approximately 1 mm of free surface for dialysis), glued with an epoxy resin to a microinjection cannula made with fused capillary silica tubing ending adjacently to the U-shaped dialysis membrane, prepared as previously described (Melis et al., 1996, 1997a,b). The probes were implanted stereotaxically (Stoelting, Wood Dale, IL, USA) into the PVN under chloral hydrate anaesthesia 2 days before the experiments (coordinates: 0.2 mm anterior to bregma, 0.4 mm lateral to midline and 2 mm ventral to dura) (Pellegrino and Cushman, 1971). The animals were given 2 days to recover from surgery, and each rat was used only once. For microinjection into the PVN, the microinjection cannula was connected by polyethylene tubing to a 10-μl Hamilton syringe driven by a Stoelting microsyringe pump. The probe was perfused with Ringer's solution containing 147 mM NaCl, 3 mM KCl and 1.2 mM $CaCl_2$, pH 6.5, at a constant flow rate of 2 μl/min by using a Stoelting 200 microsyringe pump. After a 2-h equilibration period, dialysate was collected every 20 min in fractions of 40 μl in polyethylene tubes kept at 10–15 °C for the determination of NO_2^- and NO_3^- as described below. After the collection of three dialysate aliquots, EP 80661 was dissolved in Ringer's solution and injected into the PVN in a volume of 0.3 μl. Rats were observed for 80

min, during which four additional dialysate fractions of 40 μl each were collected every 20 min, and penile erection episodes were counted. In experiments in which muscimol or morphine was used, these compounds were dissolved in saline and microinjected into the PVN in a volume of 0.3 μl over a period of 2 min, 15 min before EP 80661. When bicuculline or naloxone was used, the drugs were dissolved in saline and injected into the PVN over a period of 2 min, 5 min before muscimol or morphine, respectively.

2.4. Determination of NO_2^- and NO_3^- in the paraventricular dialysate

The concentration of NO_2^- and NO_3^- was measured in the paraventricular dialysate by a modification of the Griess reaction, as already described in detail (Melis et al., 1996, 1997a,b). Briefly, NO_2^- in the dialysate was used for the diazotization of sulfanilamide and subsequent coupling to *N*-(1-naphtyl)-ethylenediamine. The azo dye was then quantified by HPLC from its absorbance at 546 nm. The sensitivity of the assay was 0.1 μM and the response was linear with increasing concentrations of NO_2^- up to 25 μM. For the determination of NO_3^- in the dialysate, NO_3^- was previously reduced to NO_2^- with copper–cadmium, as already described (Melis et al., 1996). Total NO_2^- was then measured as described above and the NO_3^- content was calculated by subtracting that of NO_2^- found in the dialysate without copper–cadmium reduction. The sensitivity of the method was 3 μM, and the response was linear with NO_3^- up to 30 μM.

2.5. Behavioural studies

Rats were placed individually in Plexiglas cages (30 × 30 × 30 cm). After a 30-min habituation period, the microdialysis probe was connected via polyethylene tubing to a 10 μl Hamilton microsyringe driven by a Stoelting microsyringe pump on one end and to the polyethylene collecting loop on the other hand. The cannula for PVN injections was also connected to a 10-μl Hamilton microsyringe driven by a microinfusion pump via polyethylene tubing. After a 2-h equilibration period of perfusion of the dialysis probe with Ringer's solution, EP 80661 was given in the PVN over a 2-min period. When muscimol or morphine was used, these were given into the PVN 15 min before the hexarelin analogue peptide. When bicuculline or naloxone was used, these were given 5 min before muscimol or morphine, respectively. After treatments, rats were observed for the entire duration of the experiment in order to replace filled loops with empty ones every 20 min and to count penile erection episodes. Penile erections were scored when the penis emerged from the penile sheath, which was usually accompanied by penile grooming and hip flexions.

2.6. Histology

At the end of the experiments the animals were killed by decapitation, the brains were immediately removed and stored in 2% aqueous formaldehyde for 12–15 days. To localise the position of the probe tip, 50-μm transverse brain sections were prepared with a freezing microtome, stained with Neutral Red and inspected on a phase contrast microscope. The site of the probe tip was localised by following the probe tract through a series of brain sections. Only those animals found to have the probe tip positioned correctly in the PVN were considered for the statistical evaluation of the results.

2.7. Statistics

Statistical evaluation of the results was performed by analysis of variance (MANOVA). Post hoc analysis was performed by Duncan's multiple range test. A $P < .025$ was considered significant (Statistica for Windows, Release 4.0, Statsoft, 1993).

3. Results

3.1. Effect of muscimol and morphine on penile erection and on the concomitant NO_2^- and NO_3^- increase occurring in the paraventricular dialysate after EP 80661

EP 80661 (1 μg) injected into the PVN induces penile erection episodes that occur concomitantly with an increase in the concentration of NO_2^- and NO_3^- in the paraventricular dialysate two- to threefold above basal values (0.61 ± 0.17 and 4.15 ± 0.69 μM, respectively). Penile erections started within 10 min after treatment and lasted for 45–60 min. The increase in NO_2^- and NO_3^- was already observed 20 min after treatment in the first 20-μl aliquot of collected dialysate and disappeared 60 min later. Muscimol (50, 100 and 200 ng), injected into the PVN 15 min before EP 80661, reduced both penile erection episodes and the increase in NO_2^- and NO_3^- in the paraventricular dialysate. The minimal dose effective in reducing these responses significantly by about 40% was 100 ng. Maximal reduction was seen with the highest doses of the drug used, which reduced responses induced by the above substances by more than 80% (Fig. 1). MANOVA revealed a significant effect of treatment [$F(4,25) = 202.32$, 167.34 and 121.45], of time [$F(6,150) = 132.65$, 101.34 and 45.23] and a significant Treatment × Time interaction [$F(24,150) = 67.43$, 65.67 and 52.34] on penile erection, NO_2^- and NO_3^-, respectively. Post hoc analysis (Duncan's multiple range test) confirmed that EP 80661 increased penile erection, NO_2^- and NO_3^-, and that muscimol significantly reduced the EP 80661 effects ($P < .001$). At the doses used, muscimol failed to cause any marked behavioural changes.

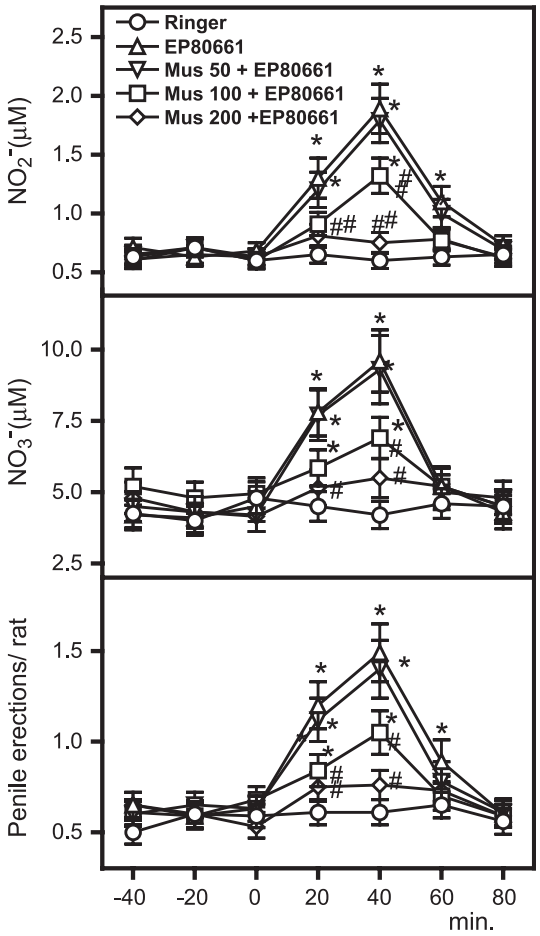

Fig. 1. Effect of muscimol on penile erection and on the concomitant NO_2^- and NO_3^- increase occurring in the paraventricular dialysate induced by EP 80661 injected into the PVN. Muscimol (50, 100 and 200 ng) dissolved in saline (0.3 μl) or saline alone was injected into the PVN 15 min before EP 80661 (1 μg) (time = 0). After injections into the PVN the animals were placed individually into Plexiglas cages and observed for 80 min during which paraventricular dialysate aliquots were collected to determine NO_2^- and NO_3^- concentration as described in Materials and Methods and penile erection episodes counted. Values are means ± S.E.M. of six rats per group. * $P < .001$ with respect to pretreatment values, # $P < .001$ with respect to the corresponding saline-pretreated group (MANOVA followed by Duncan's multiple range test).

Similar results were found when morphine (0.1, 0.5, 1 and 5 μg) was injected into the PVN. The minimal effective dose was 0.5 μg, which reduced penile erection and NO_2^- and NO_3^- increase by more than 45%. Maximal reduction was found with 5 μg, the highest dose tested, which reduced the above responses by more than 85% (Fig. 2). MANOVA revealed a significant effect of treatment [$F(5,30) = 185.12$, 172.14 and 101.41], of time [$F(6,180) = 121.15$, 121.31 and 55.25] and a significant Treatment × Time interaction [$F(30,180) = 69.41$, 55.17 and 42.14] on penile erection, NO_2^- and NO_3^-, respectively. Post hoc analysis (Duncan's multiple range test) confirmed that EP 80661 increased penile erection, NO_2^- and NO_3^-, and that morphine significantly reduced the EP 80661 effects ($P < .001$). At the doses used, morphine failed to cause any marked behavioural changes.

3.2. Effect of bicuculline on the muscimol-induced reduction of EP 80661-induced penile erection and the concomitant NO_2^- increase occurring in the paraventricular dialysate

Bicuculline (250 ng), injected into the PVN 5 min before muscimol (200 ng), prevented the inhibitory effect of muscimol on the increase of penile erection and of NO_2^- in the paraventricular dialysate induced by EP 80661 (Fig. 3). MANOVA revealed a significant effect of treatment [$F(3,20) = 151.22$ and 125.15], of time [$F(6,120) = 130.34$ and 98.45] and a significant Treatment × Time interaction [$F(18,120) = 88.23$ and 39.54] on penile erection and NO_2^-, respectively. Post hoc analysis (Duncan's multiple range test) confirmed that bicuculline prevented the inhibitory

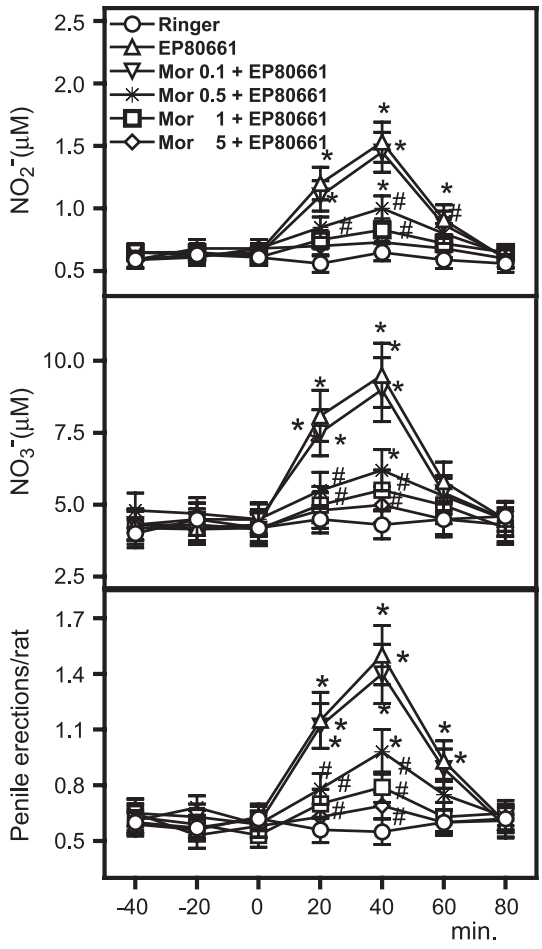

Fig. 2. Effect of morphine on penile erection and on the concomitant NO_2^- and NO_3^- increase occurring in the paraventricular dialysate induced by EP 80661 injected into the PVN. Morphine (0.5, 1 and 5 μg) dissolved in saline (0.3 μl) or saline alone was injected into the PVN 15 min before EP 80661 (1 μg) (time = 0). The other experimental conditions were identical to those described in the legend of Fig. 1. Values are means ± S.E.M. of eight rats per group. * $P < .001$ with respect to pretreatment values, # $P < .001$ with respect to the corresponding saline-pretreated group (MANOVA followed by Duncan's multiple range test).

effects of muscimol on EP 80661-induced increase of penile erection and NO_2^- ($P<.001$). At the dose used, bicuculline alone was unable to modify the basal NO_2^- concentration in the paraventricular dialysate or the NO_2^- increase or penile erections episodes induced by EP 80661 (not shown).

3.3. Effect of naloxone on the morphine-induced reduction of EP 80661-induced penile erection and the concomitant NO_2^- increase occurring in the paraventricular dialysate

Naloxone (5 μg), injected into the PVN 5 min before morphine (1 μg), prevented the inhibitory effect of morphine on the increase of penile erection and of NO_2^- in the paraventricular dialysate induced by EP 80661 (Fig. 4). MANOVA revealed a significant effect of treatment [$F(3,20)=121.22$ and 105.11], of time [$F(6,120)=120.84$ and 88.49] and a significant Treatment × Time interaction [$F(18,120)=68.23$ and 59.51] on penile erection and NO_2^-, respectively. Post hoc analysis (Duncan's multiple range test) confirmed that naloxone prevented the inhibitory effects of morphine on EP 80661-induced increase of penile erection and NO_2^- ($P<.001$). At the dose used, naloxone alone was unable to modify the basal NO_2^- concentration in the paraventricular dialysate or the NO_2^- increase or penile erections episodes induced by EP 80661 (not shown).

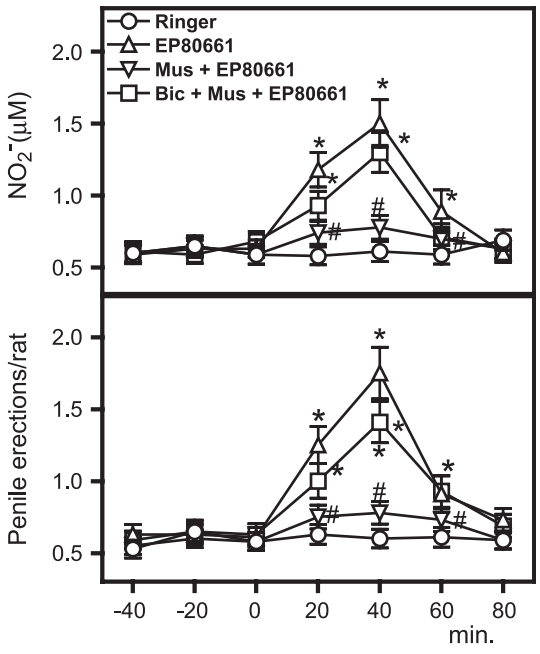

Fig. 3. Effect of bicuculline on the muscimol-induced reduction of penile erection and of the concomitant NO_2^- increase occurring in the paraventricular dialysate induced by EP 80661. Bicuculline methiodide (250 ng) dissolved in saline (0.3 μl) or saline alone was injected into the PVN 5 min before muscimol (200 ng) and 20 min before EP 80661 (1 μg) (time = 0). The other experimental conditions were identical to those described in the legend of Fig. 1. Values are means ± S.E.M. of eight rats per group. * $P<.001$ with respect to pretreatment values, # $P<.001$ with respect to the corresponding saline-pretreated group (MANOVA followed by Duncan's multiple range test).

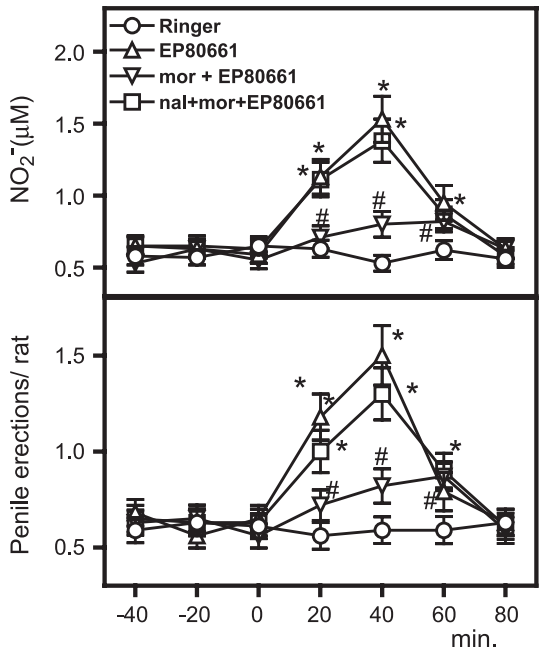

Fig. 4. Effect of naloxone on the morphine-induced reduction of penile erection and the concomitant NO_2^- increase occurring in the paraventricular dialysate induced by EP 80661. Naloxone (5 μg) dissolved in saline (0.5 μl) or saline alone was injected into the PVN 5 min before morphine (1 μg) and 20 min before EP 80661 (1 μg) (time = 0). The other experimental conditions were identical to those described in the legend of Fig. 1. Values are means ± S.E.M. of eight rats per group. * $P<.001$ with respect to pretreatment values, # $P<.001$ with respect to the corresponding saline-pretreated group (MANOVA followed by Duncan's multiple range test).

4. Discussion

The present results show that both muscimol, a $GABA_A$ receptor agonist, and morphine, a classical opioid receptor agonist, given into the PVN reduce penile erection induced by the hexarelin analogue peptide EP 80661 injected into the PVN of male rats. The inhibitory effect of muscimol and of morphine on erection occurs together with a concomitant reduction of the NO_2^- and NO_3^- increase found in the paraventricular dialysate when sexual response is induced by this peptide (Melis et al., 1996; 1997a,b). Since muscimol effect is abolished by bicuculline, a $GABA_A$ receptor antagonist, and morphine effect is reduced by naloxone, a classical opioid receptor antagonist, muscimol and morphine apparently reduce penile erection and the paraventricular NO_2^- and NO_3^- increase induced by EP 80661 by stimulating $GABA_A$ receptors and opioid receptors, respectively, in the PVN. As penile erection is apparently mediated by the activation of NO synthase present in paraventricular oxytocinergic neurons projecting to extrahypothalamic brain areas, it is likely that

these $GABA_A$ and opioid receptors are located in the cell bodies of the above oxytocinergic neurons (see Introduction). As GABAergic synapses impinge on parvocellular and magnocellular oxytocinergic neurons (Boubada et al., 1996; Jourdain et al., 1999), the finding suggests that GABAergic synapses also impinge on and control in an inhibitory fashion oxytocinergic neurons mediating penile erection by interfering with the mechanisms activated by EP 80661, which lead to the activation of NO synthase. The same also applies to opiod synapses, which impinge on and control in an inhibitory fashion the cell bodies of oxytocinergic neurons projecting not only to the neurohypophysis but also to extrahypothalamic brain areas (Muhlethaler et al., 1980; Melis et al., 1999b) In this regard, it is pertinent to recall that the molecular mechanism by which hexarelin analogue peptides activate oxytocinergic neurons mediating penile erection in the PVN is unknown at present. We have previously suggested that these peptides induce penile erection by acting on specific receptors, different from those mediating GH release or eating behaviour (both activated by hexarelin), and possibly located in the cell bodies of oxytocinergic neurons mediating penile erection and coupled to Ca^{2+} influx (see Melis et al., 2000). This would explain the peptide activation of paraventricular NO synthase found in both previous studies and in the present work. Support to this hypothesis is provided by findings showing that ω-conotoxin-GVIA, a potent blocker of N-type Ca^{2+} channels (McCleskey et al., 1987), injected into the PVN prevents hexarelin analogue peptide-induced penile erection (see Melis et al., 2000) and the concomitant increase in paraventricular NO production (Melis et al., unpublished data), as shown for dopamine receptor agonist- and oxytocin-induced penile erection (see Succu et al., 1998, and references therein). The existence of specific receptors mediating penile erection and coupled to Ca^{2+} influx for these hexarelin peptide analogues would not be particularly surprising, because specific receptors for GH-releasing peptides have been identified in the pituitary gland, the hypothalamus and other brain regions (see Pong et al., 1996; Muccioli et al., 1999; Smith et al., 1999, and references therein). Perhaps of great relevance to this work, activation of these receptors triggers GH release from the pituitary by increasing Ca^{2+} influx (Sartor et al., 1985; Akman et al., 1993; Deghenghi, 1996; Muller et al., 1999, and references therein) and experimental evidence supports the existence of different subpopulations of GH-releasing peptide receptors. First, the effects of hexarelin and its analogues on GH release are separated from the eating effects (Torsello et al., 1998). Second, receptors for hexarelin and other GH-releasing peptides, the activation of which induces effects independent of the GH-releasing properties of these peptides, have been detected in other tissues, e.g., in the heart (Bodart et al., 1999; Locatelli et al., 1999; Bisi et al., 1999). Third, cloning studies have revealed the existence of several forms of receptors for GH-releasing peptides (see Smith et al., 1999, and references therein). Finally, ghrelin, an endogenous acylated peptide agonist of these receptors, mainly of those that induce GH release and feeding behaviour, has recently been isolated from stomach (Kojima et al., 1999). This endogenous GH-releasing peptide is also expressed in different tissues, including the central nervous system and in the hypothalamus (Hosoda et al., 2000). Interestingly, ghrelin injected into the PVN induces feeding but not penile erection, in line with the hypothesis that receptors mediating the proerectile effect of hexarelin analogue peptides are different from those mediating feeding behavior and/or GH release (Melis et al., 2002).

The ability of muscimol and morphine to reduce penile erection induced by hexarelin analogue peptides and the concomitant paraventricular NO_2^- and NO_3^- increase that occurs in the PVN resemble the ability of these compounds to reduce such responses when induced by drugs as apomorphine, oxytocin and NMDA, or when they occur in physiological contexts, as when sexually potent male rats are put in the presence of an inaccessible receptive female (noncontact erections) or during copulation (Melis and Argiolas, 2002; Melis et al., 1999b, and references therein). Indeed, (1) apomorphine, oxytocin and NMDA also induce penile erection and increase concomitantly NO production in the PVN by acting on specific receptors coupled to an increased Ca^{2+} influx into the cell bodies of oxytocinergic neurons mediating this sexual response, (2) an increase in NO production occurs in the PVN during noncontact erections and copulation (see Melis et al., 1998) and (3) these responses are reduced either by muscimol or by morphine given into the PVN (Melis and Argiolas, 2002; Melis et al., 1999a,b). Although the exact mechanism of action of muscimol and of morphine occurring in the PVN in preventing these responses induced by hexarelin analogue peptides is still unknown, the fact that both muscimol and morphine prevent penile erection and paraventricular NO_2^- and NO_3^- increase induced by all the above compounds, confirms that GABA and endogenous opioids exert an inhibitory control on oxytocinergic neurons mediating such responses. Accordingly, GABA inhibits oxytocin release induced by suckling and by osmotic stimuli (see Bisset et al., 1990; Voisin et al., 1995), and morphine inhibits oxytocin release stimulated not only by suckling, but also by parturition or by electrical stimulation of the neurohypophyseal system in vitro and in vivo (Clarke et al., 1979; Pittman et al., 1980; Muhlethaler et al., 1980, Bicknell et al., 1988) by acting on opioid receptors of the μ subtype (Wuarin et al., 1988).

Although further studies are required to clarify the mechanism by which muscimol and morphine reduce hexarelin analogue peptide-induced penile erection, this study shows that $GABA_A$ receptors and opioid receptors in the PVN inhibit penile erection induced by hexarelin analogue peptides. Interestingly, this inhibition is mediated by a decrease of the NO synthase activation that occurs

during sexual response in the above hypothalamic nucleus, as found when the sexual response is induced by other proerectile agents that act in the PVN, or when it occurs in physiological contexts.

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