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ORIGINAL ARTICLE

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Prevention by morphine of apomorphine- and oxytocin-induced penile erection and yawning: involvement of nitric oxide

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Abstract The possible involvement of nitric oxide in the prevention by morphine of apomorphine- and oxytocin-induced penile erection and yawning was investigated by measuring the concentration of NO_2^- and NO_3^- in the dialysate obtained with a vertical microdialysis probe implanted in the paraventricular nucleus of the hypothalamus of male rats. Either apomorphine (80 µg/kg s.c.) or oxytocin (30 ng i.c.v.) increased significantly basal NO_2^- and NO_3^- concentration in the paraventricular dialysate, penile erection and yawning. Morphine (1, 5 and 10 mg/kg i.p.) prevented dose-dependently either apomorphine or oxytocin responses when given 15 min before apomorphine or oxytocin. Prevention by morphine of apomorphine and oxytocin responses was abolished by naloxone (3 mg/kg i.p.) given 15 min before morphine. Morphine prevented apomorphine and oxytocin responses also when given in the lateral ventricles or directly in the paraventricular nucleus. In contrast, the selective agonist of the kappa opioid receptor subtype U-69,593 was found to be ineffective. The present results confirm previous findings showing that morphine acts through µ receptors in the paraventricular nucleus to prevent apomorphine and oxytocin-induced penile erection and yawning and suggest that this morphine effect is mediated by a decreased activity of nitric oxide in the paraventricular nucleus of the hypothalamus.

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Introduction

Penile erection and yawning are two different behavioral patterns that often occur concomitantly in different experimental conditions (see Holmgreen et al. 1985; Meisel and Sachs 1994; Argiolas and Gessa 1991; Argiolas and Melis 1995; Melis and Argiolas 1995a). While the importance of penile erection in reproduction does not need to be stressed, it is pertinent to recall that yawning, alone or associated with stretching, is considered an ancestral vestige surviving through evolution that subserves the purpose of arousal (see Bertolini and Gessa 1981). Dopamine receptor agonists (i.e. apomorphine) (see Melis and Argiolas 1995a) and oxytocin (see Argiolas and Gessa 1991) are among the most studied substances that induce both penile erection and yawning. Both compounds induce these behavioral responses by acting in the paraventricular nucleus of the hypothalamus (PVN) apparently by activating central oxytocinergic transmission. Accordingly, these dopamine agonist- and oxytocin-induced responses are prevented by electrolytic lesions of the PVN, which deplete central oxytocin, and by oxytocin receptor antagonists given into the lateral ventricles (see Melis et al. 1992a and references therein).

Recently, we found that the induction of penile erection and yawning by dopamine receptor agonists and oxytocin depends on a normal activity of nitric oxide (NO) synthase in the PVN, because the inhibition of this enzyme in this nucleus, but not in other brain areas prevents these behavioral responses induced either by apomorphine or oxytocin (Melis et al. 1994). Interestingly the PVN is one of the richest brain areas containing NO synthase, which among others has been identified also in the cell bodies of oxytocinergic neurons (Bredt et al. 1990; Vincent and Kimura 1992; Sanchez et al. 1994;

Torres et al. 1993). The involvement of NO in the induction of penile erection and yawning by dopamine receptor agonists and by oxytocin has been recently substantiated by showing that either apomorphine- or oxytocin-induced penile erection and yawning are associated to an increase of the concentration of NO_2^- and NO_3^-, the reaction products of newly synthetized NO with O_2 which are an indirect but reliable indicator of NO production by biological tissues in vivo (see Ignarro 1990; Ohta et al. 1994; Melis et al. 1996), in the dialysate collected from a vertical microdialysis probe implanted in the PVN (Melis et al. 1996, 1997). Since apomorphine- and oxytocin-induced penile erection and yawning are prevented also by the opiate morphine given systemically or directly into the PVN (Melis et al. 1992b), we studied whether this effect of the opiate on penile erection and yawning induced by apomorphine and oxytocin was related to changes of the NO production in the PVN of male rats by in vivo microdialysis.

Material and methods

Animals. Male Sprague Dawley rats (200-220 g) (Charles River, Como, Italy) were used in all the experiments. Animals were caged in groups of 4-6 at 24 C, humidity 60%, lights on from 7 to 19 h with water and standard laboratory food ad libitum. All experiments were performed between 9-13 h.

Drugs, peptides and reagents. Morphine-HCl, naloxone-HCl, apomorphine-HCl, sulfanilamide and N-(1-naphtyl)-ethylenediamine were purchased from Sigma (St. Louis, MO, USA), oxytocin from Peninsula Eur. Ltd. (St. Helens/Meyerside, UK), U-69,593 [(+)-5α, 7α, 8β)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspirol[4, 5] dec-8-yl]-benzene-acetamide] from Research Biochemicals International (Natick, MA, USA). All the drugs, peptides and reagents were of the highest available purity.

Systemic treatments. Naloxone and morphine were dissolved with saline and injected intraperitoneally (i.p.) in a volume of 0.5 ml/100 g body weight. Apomorphine was dissolved with saline and injected subcutaneously (s.c.) in a volume of 0.1 ml/100 g body weight in the back of the neck. Controls received the same volume of saline either i.p. or s.c.

I.C.V. injections. For i.c.v. injections, cannulas were constructed in our laboratory as already described (Melis et al. 1996). Briefly, fused silica capillary tubing (out diameter 0.15 mm) was inserted into a piece of a 22 gauge stainless tubing extending approximately 3 mm beyond the tip of the stainless tubing. The silica and stainless tubings were then glued each other with waterproof epoxy resin. The cannulas aimed at one of the lateral ventricles (coordinates 1 mm anterior to bregma, 1 mm lateral to midline and 3 mm vertical from the dura) (Pellegrino and Cushman 1971) were implanted stereotaxically (David Kopf Instruments) under anesthesia with chlorale hydrate (Sigma, 350 mg/kg i.p.) during the implantation of the microdialysis probe (see below). Oxytocin, morphine or U-69,593 dissolved in saline or saline alone was injected in a volume of 10 µl.

PVN microinjections and in vivo microdialysis. In order to perform PVN microinjections and PVN microdialysis in the same animal microdialysis probes, with approximately 1 mm of free surface for dialysis (out diameter 0.28 mm), using a loop flow design were prepared as already described (Melis et al. 1996 and references therein) except that an infusion cannula made with fused capillary silica tubing (out diameter 0.15 mm) ending adjacent to the U-shaped membrane was glued to the microdialysis probe with epoxy resin. The modified probes were then implanted in the PVN (coordinates 0.2 mm anterior to the bregma, 0.4 mm lateral to the midline, and 7.3 mm vertical from the dura) (Pellegrino and Cushman 1971) under chlorale hydrate anesthesia, two days before the experiments. Each rat was used only once. The probes were 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 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 tubing loops and transferred in polyethylene tubes at a temperature of 10-15 C for the determination of NO_2^- and NO_3^- as described below. Ringer's solution (0.3 µl) alone or containing morphine or U69,593 was injected in the PVN over a period of 2 min 15 min before s.c. apomorphine or i.c.v. oxytocin.

Determination of NO_2^- and NO_3^- concentration. NO_2^- concentration in the dialysate was determined by a modification of the Griess reaction as already described (Melis et al. 1996). Briefly, NO_2^- in the dialysate was used for the diazotization of sulfanilamide and subsequent coupling to N-(1-naphtyl)-ethylenediammine. The azo dye was then quantified by high pressure liquid cromatography (HPLC) from its absorbance at 546 nm with a Waters LC Module I cromatograph equipped with a UV 486 detector, a WISP 715 autoinjector and a 0.4×15 cm Novapak C18 column (Waters Ass.). The sensitivity of the assay was 0.1 µM equivalent to about 0.3 ng of $NaNO_2$ in 40 µl of dialysate and the response was found to be linear with increasing concentrations of NO_2^- up to 25 µM. For the determination of NO_3^- in the dialysate, NO_3^- was reduced to NO_2^- with copper-cadmium (see Melis et al. 1996) and total NO_2^- was then determined as described above. The amount of NO_3^- was then calculated by subtracting that of NO_2^- found in the aliquot of dialysate without copper-cadmium reduction. The sensitivity of the method was 3 µM (10 ng of $NaNO_3$ in 40 µl of dialysate) and the response was linear with NO_3^- up to 30 µM.

Behavioral 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 the Stoelting microsyringe pump on one end and to the polyethylene collecting loop on the other hand. The cannula for i.c.v. or PVN injections was also connected to a microinfusion pump driven by hand or by a Stoelting microinfusion pump, respectively, via polyethylene tubing. After a 2 h equilibration period of perfusion of the dialysis probe with Ringer's solution, apomorphine was given s.c. and oxytocin i.c.v. over a 2 min period. In those experiments in which morphine or U-69,593 was given, the former was given i.p., i.c.v. or into the PVN and the latter only i.c.v. or into the PVN 15 min before apomorphine or oxytocin. When naloxone was used, this was given i.p. 15 min before morphine and 30 min before apomorphine or oxytocin. After treatments, rats were observed for 2 h to replace filled loops with empty ones every 20 min and to count penile erection and yawning episodes.

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 10-12 days. To localize the position of the probe tip, 50 µm transverse brain sections were prepared by means of a freezing microtome, stained with Neutral Red and inspected on a phase contrast microscope. The site of the probe tip was localized 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 (see below) were considered for the statistical evaluation of the results.

Statistics. Statistical evaluation of the results was performed by analysis of variance (one-way ANOVA), followed by Duncan's multiple range test. A $P < 0.05$ was considered significant (Tallarida and Murray 1986).

Results

In agreement with previous studies, apomorphine (80 µg/kg s.c.) given after a 2 h equilibration period, increased the concentration of NO_2^- from 0.95 ± 0.29 µM to a maximum of 4.5 ± 0.69 µM and NO_3^- from 5.6 ± 0.85 to 11.25 ± 1.90 µM in the paraventricular dialysate ($P < 0.01$) (Fig. 1). These increases were correlated to the increase in the number of penile erection and yawning episodes induced by the drug. As expected, morphine (5 and 10 but not 1 mg/kg i.p.) prevented apomorphine-induced penile erection and yawning when given 15 min before apomorphine ($P < 0.01$). Most important, the inhibitory effect of morphine on the behavioral responses was correlated with an almost complete prevention of the increase of NO_2^- and NO_3^- induced by apomorphine in the paraventricular dialysate ($P < 0.01$). In contrast, the dose of 1 mg/kg, which was unable to prevent penile erection and yawning, was also unable to prevent the NO_2^- and NO_3^- increase ($P > 0.1$, not significant). Despite its ability to prevent the apomorphine-induced increase of NO_2^- and NO_3^- in the dialysate, the highest dose of morphine tested was unable to modify baseline levels of NO_2^- and NO_3^- in the paraventricular dialysate (Fig. 1).

The inhibitory effect of morphine (5 mg/kg i.p.) on the apomorphine-induced NO_2^- increase in the paraventricular dialysate, penile erection and yawning was prevented by naloxone (3 mg/kg i.p) given 15 min before morphine (Fig. 2). At the dose used, naloxone given

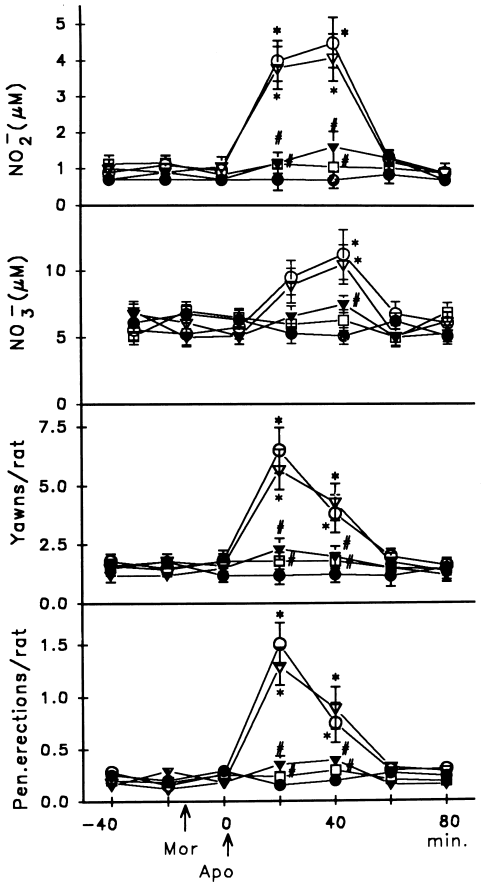

Fig. 1 Effect of morphine on the increase of NO_2^- and NO_3^- concentration in the paraventricular dialysate, penile erection and yawning induced by apomorphine. Rats were placed individually into a Plexiglas cage and perfused with Ringer solution as described in the materials and methods section. Apomorphine (80 µg/kg s.c.) was given after a 120 min equilibration period of the probe with the perfusion buffer (time = 0). The perfusion rate was 2 µl/min during the experiment. Aliquots of 40 µl were collected every 20 min and analyzed for NO_2^- and NO_3^- content. Morphine (1, 5 and 10 mg/kg i.p.) was given 15 min before apomorphine. ❍ represent apomorphine-treated rats; ● 10 mg/kg morphine-treated rats; Δ 1 mg/kg morphine + apomorphine-treated rats; ♦ 5 mg/kg morphine + apomorphine-treated rats; squares 10 mg/kg morphine + apomorphine-treated rats. During perfusion, the animals were observed in order to count penile erection and yawning episodes. Each value is the mean ± SEM of 12 rats. * $P < 0.01$ with respect to pretreatment values; # $P < 0.01$ with respect to the corresponding values of apomorphine-treated rats (One-way ANOVA followed by Duncan's multiple range test)

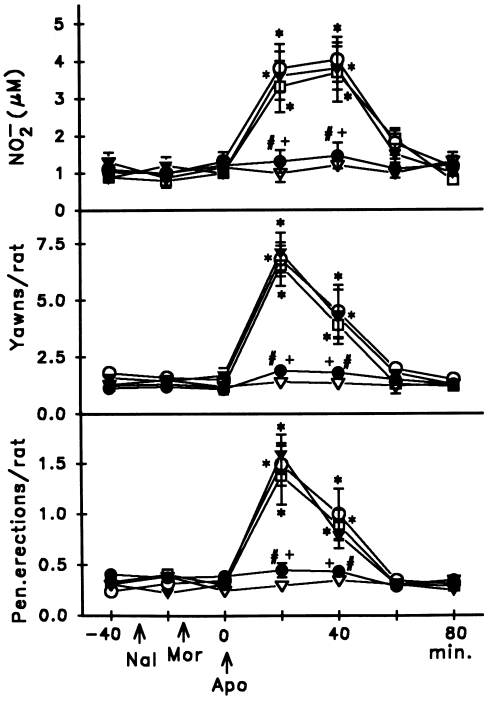

Fig. 2 Prevention by naloxone of morphine-induced prevention of apomorphine effect on paraventricular NO_2^-, penile erection and yawning. Naloxone (3 mg/kg i.p.) was given 15 min before morphine (5 mg/kg i.p.), that was given 15 min before apomorphine (80 µg/kg s.c.). ❍ represent apomorphine-treated rats; ● morphine + apomorphine-treated rats; Δ naloxone-treated rats; ♦ naloxone + morphine + apomorphine-treated rats; empty squares naloxone + saline + apomorphine-treated rats. The other experimental conditions were identical to those reported in the legend of Figure 1. During perfusion, the animals were observed in order to count penile erection and yawning episodes. Each value is the mean ± SEM of 9 rats. * $P < 0.01$ with respect to pretreatment values; # $P < 0.01$ with respect to the corresponding values of apomorphine-treated rats; + $P < 0.01$ with respect to the corresponding values of naloxone + apomorphine-treated rats (One-way ANOVA followed by Duncan's multiple range test)

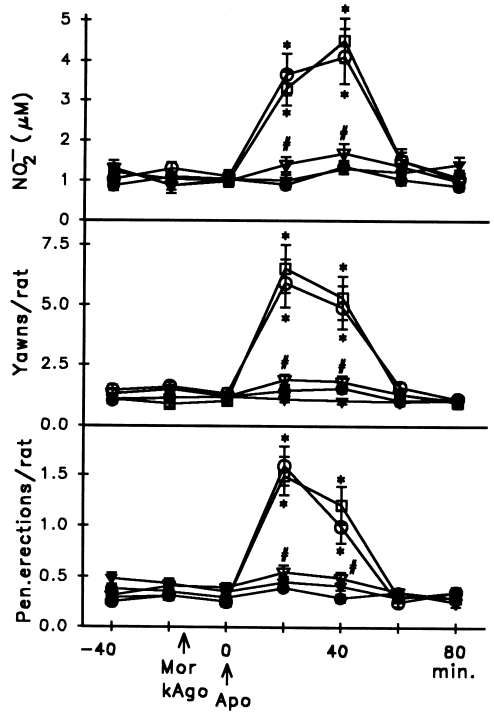

Fig. 4 Effect of morphine and U-69,593 injected into the PVN on apomorphine-induced NO_2^- increase in the paraventricular dialysate, penile erection and yawning. Morphine (2 μg) or U-69,593 (5 μg) was given into the PVN 15 min before apomorphine (80 μg/kg s.c.). ❍ represent apomorphine-treated rats; ● morphine-treated rats; Δ morphine + apomorphine-treated rats; ♦ U-69,593-treated rats; ❑ U-69,593 + apomorphine-treated rats. During perfusion, the animals were observed in order to count penile erection and yawning episodes. Each value is the mean ± SEM of 8 rats. * $P < 0.01$ with respect to pretreatment values; # $P < 0.01$ with respect to the corresponding values of apomorphine-treated rats (One-way ANOVA followed by Duncan's multiple range test)

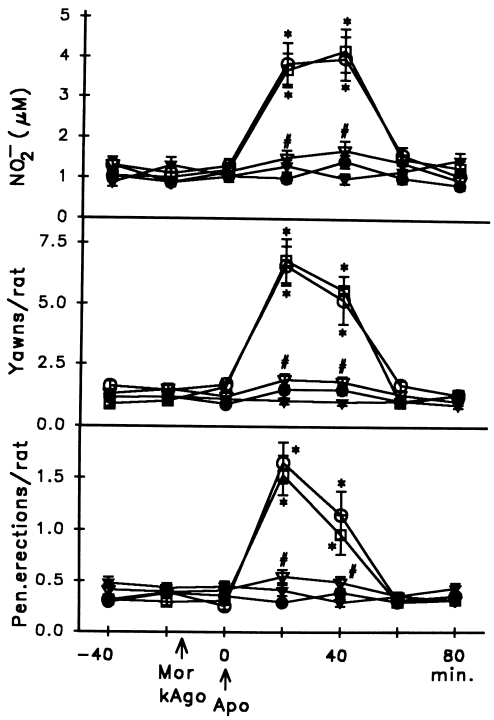

Fig. 3 Effect of morphine and U-69,593 injected i.c.v. on apomorphine-induced NO_2^- increase in the paraventricular dialysate, penile erection and yawning. Morphine (10 μg) or U-69,593 (20 μg) was given i.c.v. 15 min before apomorphine (80 μg/kg s.c.). ❍ represent apomorphine-treated rats; ● morphine-treated rats; Δ morphine + apomorphine-treated rats; ♦ U-69,593-treated rats; ❑ U-69,593 + apomorphine-treated rats. During perfusion, the animals were observed in order to count penile erection and yawning episodes. Each value is the mean ± SEM of 8 rats. * $P < 0.01$ with respect to pretreatment values; # $P < 0.01$ with respect to the corresponding values of apomorphine-treated rats (One-way ANOVA followed by Duncan's multiple range test)

Table 1 Effect of morphine given i.p. on oxytocin-induced increase of NO_2^- and NO_3^- concentration in the paraventricular dialysate, penile erection and yawning

Pretreatment (mg/kg i.p.)	Treatment	NO_2^- (ng i.c.v.)	NO_3^- (μM)	Penile (μM)	Yawns/rat erections/rat
Saline (1 ml)	Saline(10 μl)	1.1 ± 0.3	5.6 ± 0.9	0.4 ± 0.1	1.8 ± 0.4
Saline (1 ml)	Oxytocin (30)	4.2 ± 0.3*	11.3 ± 1.9*	4.2 ± 0.1*	18.0 ± 2.3*
Morphine (1)	Oxytocin (30)	4.6 ± 0.5*	10.5 ± 2.1*	3.6 ± 0.5*	17.0 ± 2.2*
Morphine (5)	Oxytocin (30)	1.9 ± 0.5*,#	7.6 ± 1.7*,#	1.5 ± 0.5*,#	7.0 ± 2.1*,#
Morphine (10)	Oxytocin (30)	1.0 ± 0.4#	6.0 ± 1.1#	0.6 ± 0.2#	1.8 ± 2.0#
Naloxone (3)	Oxytocin (30)	3.9 ± 0.4*	10.5 ± 2.0*	3.8 ± 0.6*	17.7 ± 2.1*
Naloxone (3) Morphine (5)	Oxytocin (30)	4.1 ± 0.6*,+	12.0 ± 2.1*,+	4.0 ± 0.1*,+	17.2 ± 0.2*,+

(The experimental conditions were identical to those reported in the legend of Fig. 1. During perfusion, the animals were observed in order to count penile erections and yawns. Naloxone was given 30 min and morphine 15 min before oxytocin. NO_2^- and NO_3^- values are the mean ± SEM of the maximal values found in the dialysate of 5 rats during the time course experiments, while penile erections and yawns values are the mean ± SEM of the penile erections and yawns counted in these rats after oxytocin treatment.
*$P < 0.01$ with respect to saline-treated rats; #$P < 0.01$ with respect to oxytocin-treated rats;
+$P < 0.01$ with respect to the corresponding group of morphine-treated rats (Oneway ANOVA followed by Duncan's multiple range)

alone after a 2 h equilibration period was unable per se to modify baseline levels of NO_2^-, penile erection and yawning as well as apomorphine-induced responses (Fig. 2).

Morphine was found to be effective on apomorphine effects also when given centrally. Indeed 10 μg prevented the apomorphine-induced increase of NO_2^- in the paraventricular dialysate, penile erection and yawning

Table 2 Effect of morphine and U-69,593 given i.c.v. or into the PVN on oxytocin-induced increase of NO_2^- concentration in the paraventricular dialysate, penile erection and yawning

Pretreatment	Treatment (ng i.c.v.)	NO_2^- (µM)	Penile erections/rat	Yawns/rat
Saline (10 µl i.c.v.)	Saline(10 µl)	1.0 ± 0.3	0.4 ± 0.1	1.5 ± 0.4
Saline (10 µl i.c.v.)	Oxytocin (30)	4.4 ± 0.3*	3.7 ± 0.5*	19.0 ± 2.5*
Morphine (10 µg i.c.v.)	Oxytocin (30)	1.2 ± 0.2#	0.3 ± 0.1#	1.2 ± 0.2#
U-69,593 (20 µg i.c.v.)	Oxytocin (30)	3.8 ± 0.3*	3.9 ± 0.8*	17.3 ± 1.3*
Saline (0.3 µl in the PVN)	Oxytocin (30)	4.0 ± 0.3*	3.5 ± 0.4*	17.0 ± 2.5*
Morphine (2 µg in the PVN)	Oxytocin (30)	0.8 ± 0.4#	0.6 ± 0.5*	2.0 ± 0.4#
U-69,593 (5 µg in the PVN)	Oxytocin (30)	3.6 ± 0.5*	3.3 ± 0.6*	16.8 ± 2.2*

Experimental conditions were as reported in the legend of Fig. 1. During perfusion, rats were observed in order to count penile erections and yawns. I.c.v. or PVN pretreatments were done 15 min before oxytocin. NO_{2^-} values are the mean ± SEM of the maximal NO_2^- value found in the dialysate of 6 rats during the time course experiments, while penile erections and yawns values are the mean ± SEM of the penile erections and yawns counted in these rats after oxytocin treatment.
*$P < 0.01$ with respect to saline-pretreated rats; #$P < 0.01$ with respect to the corresponding group of oxytocin-treated rats (Oneway ANOVA followed by Duncan's multiple range test)

when given i.c.v. 15 min before apomorphine ($P < 0.01$) (Fig. 3). In contrast, the kappa opioid receptor agonist U-69,593 (20 µg i.c.v.) was ineffective ($P > 0.01$). Similar results were found when 2 µg of morphine or 5 µg of U-69,593 were injected directly in the PVN (Fig. 4). Morphine and U-69,593 were both unable per se to modify baseline levels of NO_2^- in the paraventricular dialysate when given i.c.v. (Fig. 3) or into the PVN (Fig. 4).

Results similar to those found with apomorphine were obtained when penile erection and yawning were induced by oxytocin (30 ng i.c.v.), which increased the concentration of NO_2^- from 1.10 ± 0.25 µM to 4.2 ± 0.65 µM and of NO_3^- from 5.6 ± 0.9 µM to 11.3 ± 1.9 µM in the paraventricular dialysate ($P < 0.01$) (Table 1). In these experiments also morphine (5 and 10 mg/kg i.p.) prevented both the NO_2^- and NO_3^- increase and the behavioral responses, while 1 mg/kg was ineffective (Table 1). As found with apomorphine, naloxone (3 mg/kg i.p.) given 15 min before morphine, prevented both the morphine prevention of NO_2^- increase and the behavioral responses induced by oxytocin ($P < 0.01$) (Table 1). Likewise, morphine (10 µg i.c.v. or 2 µg into the PVN) but not U-69,593 (20 µg i.c.v. or 5 µg into the PVN) prevented the oxytocin responses when given 15 min before oxytocin (Table 2).

Discussion

The present results show that morphine given either systemically or i.c.v. or into the PVN prevents the increase of the concentration of NO_2^- and NO_3^-, induced by the dopamine receptor agonist apomorphine or by oxytocin, in the paraventricular dialysate obtained from male rats implanted with a vertical microdialysis probe. In agreement with previous findings showing that both apomorphine and oxytocin, at the doses used in this study, activates NO synthase in the PVN to induce these behavioral responses (see Introduction and Melis et al. 1996 and references therein), the prevention by morphine of the NO_2^- and NO_3^- increase is correlated to the well known prevention by the opiate injected in the PVN of penile erection and yawning induced by the above compounds (Melis et al. 1992b). The effect of morphine is mediated by the stimulation of opioid receptors, being prevented by the prior administration of the opioid receptor antagonist naloxone (Melis et al. 1992b). Since U-69,593, an opioid receptor agonist that is 500 times more selective than morphine on the kappa opioid receptor subtype (Lahti et al. 1985), is ineffective in preventing apomorphine and oxytocin responses either when injected i.c.v. or into the PVN, these findings suggest that morphine prevents the activation of NO synthase, penile erection and yawning induced by apomorphine or oxytocin by acting on opioid receptors of the µtype located in the PVN. It is likely that these receptors are located in the cell bodies of parvocellular oxytocinergic neurons projecting to extrahypothalamic brain areas and mediating these behavioral responses (see Melis et al. 1992a; Argiolas and Melis 1995). Accordingly, inhibitory µ opioid receptors are present in the cell bodies of paraventricular oxytocinergic neurons (Muhlethaler et al. 1980; Pittman et al. 1980; Wuarin et al. 1988 and references therein).

In line with the hypothesis discussed above, morphine might then prevent NO production in the PVN by acting on opioid receptors, whose activation interfere with the mechanisms responsible for the activation of NO synthase by apomorphine and oxytocin. In this regard, it is pertinent to recall that 1) the prevention by morphine of the NO_2^- and NO_3^- increase in the PVN dialysate would reflect almost exclusively a decreased conversion of L-arginine to NO that is in turn oxidized mainly to NO_2^-, and to a lesser extent to NO_3^-, as found in other biological fluids not containing blood cells (see Marletta et al. 1988; Ignarro 1990; Butler et al. 1995; Ohta et al. 1994), 2) the PVN is one of the richest brain areas in NO synthase, and 3) the enzyme has been localized in the cell bodies of oxytocinergic neurons (Bredt et al. 1990; Vincent and Kimura 1992; Torres et al. 1993; Sanchez et al. 1994). Since the blockade of N-type voltage dependent calcium channels by nanogram amounts of omega-conotoxin injected in the PVN prevents penile erection and yawning induced either by apomorphine or by oxytocin (Argiolas et al. 1990), it is tempting to speculate that

opioid receptors are negatively coupled to Ca^{2+} channels and that their stimulation by morphine leads to a decline in Ca^{2+} influx which in turn causes a decrease in the activity of NO synthase, a calcium-calmodulin dependent enzyme (see Snyder 1992 and enclosed references) in the cell bodies of oxytocinergic neurons medianting penile erection and yawning. The decreased NO formation would in turn reduce the oxytocinergic transmission by a yet unknown mechanism, apparently unrelated to the activation of guanylate cyclase (see Melis and Argiolas 1995b), decreasing thereby the behavioral responses. However, the present results do not rule out other mechanisms by means of which morphine might interfere with NO synthase activation induced by apomorphine or oxytocin. For instance morphine might inhibit directly NO synthase or alter indirectly its activity in oxytocinergic neurons by modulating the release of other neurotransmitters/neuromodulators in the PVN. However, the first possibility is unlikely, because 1) morphine alone does not cause any decrease in basal NO_2^- and NO_3^- levels (this study), and 2) acute or chronic morphine does not alter NO synthase activity in brain homogenates nor its expression, as measured by the amount of NO-synthase mRNA in several mouse brain areas (Babey et al. 1994).

Interactions between opioids and NO have been shown to occur in other central functions. For instance NO seems to be involved in the expression of opioid antinociception, tolerance and dependence, although with some discrepancy (see Babey et al. 1994; Dambisya and Lee 1996 and references therein). In these studies either facilitatory or inhibitory effects of NO on morphine effects were found. The present results show that morphine and NO interact in opposite manner in the control of penile erection and yawning at paraventricular level. In particular the findings suggest that μ opioid receptor stimulation in the PVN decreases NO synthase activity to inhibit these behavioral responses induced by dopamine receptor agonists or by oxytocin.

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