ELSEVIER

European Journal of Pharmacology 294 (1995) 1–9

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Nitric oxide donors induce penile erection and yawning when injected in the central nervous system of male rats

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Received 2 March 1995; revised 7 August 1995; accepted 11 August 1995

Abstract

In order to provide further support for a role of central nitric oxide as a mediator of penile erection and yawning, the nitric oxide donors sodium nitroprusside, hydroxylamine, isoamyl nitrite and *S*-nitroso-*N*-acetyl-penicillamine were injected into the lateral ventricles (i.c.v.) or into the paraventricular nucleus of the hypothalamus of male rats. Of the above compounds injected i.c.v., only isoamyl nitrite (10–100 μg) induced penile erection and yawning, while the others induced dramatic behavioral changes, such as motor hyperactivity and convulsions, that masked the above responses. Nevertheless, nitric oxide donors in doses ranging from 10 to 50 μg, for except *S*-nitroso-*N*-acetyl-penicillamine that was injected only at the dose of 10 μg and isoamyl nitrite that was not injected at all because of poor solubility, induced penile erection and yawning when injected in the paraventricular nucleus. Nitric oxide donor-induced responses were prevented by methylene blue and LY 83583, inhibitors of guanylate cyclase, the best known target of nitric oxide, given i.c.v. but not in the paraventricular nucleus. However, 8-bromo-guanosine 3′:5′-cyclic monophosphate (8-Br-cGMP), a stable cGMP analog, and hemoglobin, a nitric oxide scavenger, were ineffective in inducing and preventing, respectively, penile erection and yawning when injected either i.c.v. or in the paraventricular nucleus. Nitric oxide donor-induced responses were also prevented by the nonapeptide oxytocin receptor antagonist $d(CH_2)_5$-Tyr(Me)-Orn^8-vasotocin given i.c.v. but not in the paraventricular nucleus. The present results suggest that nitric oxide donors induce penile erection and yawning by activating central oxytocinergic transmission in the paraventricular nucleus of the hypothalamus via a cGMP-independent mechanism.

Keywords: Nitric oxide (NO) donor; Penile erection; Yawning; Guanylate cyclase; Paraventricular nucleus; (Rat)

1. Introduction

Penile erection and yawning are two different behavioral patterns that often occur concomitantly in physiological and experimental conditions (Holmgren et al., 1985). While the importance of penile erection in reproduction does not need to be stressed (for a review see Sachs and Meisel, 1988), it is pertinent to recall that yawning alone or associated with stretching is considered a vestigial behavior that has survived through evolution and which subserves the purpose of arousal (see Bertolini and Gessa, 1981). Among substances that induce both these behavioral responses, the best known are dopamine receptor agonists (see Melis and Argiolas, 1995), oxytocin (see Argiolas and Gessa, 1991), adrenocorticotropin (ACTH) and related peptides (see Bertolini and Gessa, 1981). NMDA (Roeling et al., 1991; Melis et al., 1994a) and 5-HT receptor agonists that act mainly on the 5-HT_{1c} receptor subtype (see Stancampiano et al., 1994 and enclosed references). Several lines of evidence suggest that the paraventricular nucleus of the hypothalamus plays a key role in the expression of the behavioral responses induced by dopamine receptor agonists, NMDA and oxytocin, but not by ACTH or 5-HT receptor agonists. In particular, it seems that dopamine receptor agonists, NMDA and oxytocin itself induce the behavioral responses by activating oxytocinergic transmission. Accordingly, (1) dopamine receptor agonists, oxytocin and NMDA induce penile erection and yawning when injected in this hypothalamic nucleus (Melis et al., 1992, 1994a), while ACTH and 5-HT receptor agonists are ineffective (Argiolas et al., 1990;

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SSDI 0014-2999(95)00508-0

Stancampiano et al., 1994); (2) dopamine receptor agonist-, NMDA- and oxytocin- but not ACTH- or 5-HT_{1c} receptor agonist-induced penile erection and yawning are prevented by the central administration of oxytocin receptor antagonists (Argiolas and Gessa, 1991; Melis et al., 1992, 1994a; Stancampiano et al., 1994) and by electrolytic lesions of the paraventricular nucleus of the hypothalamus (Argiolas and Gessa, 1991), which induce an almost complete depletion of the oxytocin content in extra-hypothalamic brain areas and spinal cord (Lang et al., 1983; Hawthorn et al., 1985).

Recently, we provided experimental evidence that nitric oxide, the novel transmitter/modulator present in several tissues including brain (see Moncada et al., 1989; Ignarro, 1990, Snyder, 1992; Schuman and Madison, 1994), plays an important role in the expression of penile erection and yawning induced by apomorphine, a classical dopamine receptor agonist, NMDA, oxytocin and 5-HT_{1c} receptor agonists. Accordingly, (1) inhibitors of nitric oxide-synthase, a calmodulin-dependent iron-containing enzyme that produces nitric oxide from L-arginine (Moncada et al., 1989; Ignarro, 1990; Snyder, 1992), prevent the behavioral responses induced by apomorphine, oxytocin, NMDA and 5-HT_{1c} receptor agonists when injected into the lateral ventricles (i.c.v.) (Melis and Argiolas, 1993; Argiolas, 1994; Melis et al., 1994c, 1995b); (2) the central administration of nitroglycerin, a putative nitric oxide donor (see Murad, 1990), induces penile erection and yawning apparently by increasing central oxytocinergic transmission (Melis et al., 1995a); and (3) the paraventricular nucleus of the hypothalamus seems to be one of the areas where nitric oxide controls the behavioral responses induced by all the above agents (Melis et al., 1994b,c, 1995a) except 5-HT_{1c} receptor agonists (Melis et al., 1995b). In the present study we further investigated the involvement of nitric oxide in the control of these behavioral responses by studying the central effect of other classic nitric oxide donors, such as sodium nitroprusside, *S*-nitroso-*N*-acetyl-penicillamine, hydroxylamine and isoamyl nitrite, on spontaneous penile erection and yawning. Furthermore, since nitric oxide is thought to act by activating guanylate cyclase, the effect of methylene blue and LY 83583, putative guanylate cyclase inhibitors (Murad et al., 1978; Gruetter et al., 1981, Mülsch et al., 1988), and of hemoglobin, a nitric oxide scavenger (Murad et al., 1978) on nitric oxide donor-induced penile erection and yawning as well as the effect of 8-bromo-guanosine 3′:5′-cyclic monophosphate (8-Br-cGMP), a metabolically stable analog of cGMP, on spontaneous penile erection and yawning were studied. Finally, the effect of a potent nonapeptide oxytocin receptor antagonist, d$(CH_2)_5$-Tyr(Me)-Orn^8-vasotocin (Bankowski et al., 1980), on nitric oxide donor-induced penile erection and yawning is also reported.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (200–220 g) (Morini, S. Polo d'Enzo, Italy) were used in all the experiments. Animals were caged in groups of four to six at 24°C, humidity 60%, lights on from 07:00 to 19:00 h with water and standard laboratory food ad libitum. All experiments were performed between 09:00–13:00 h.

2.2. Drugs and peptides

Sodium nitroprusside, isoamyl nitrite, hydroxylamine-HCl, 8-bromo-guanosine 3′:5′-cyclic monophosphate (8-Br-cGMP), methylene blue, and rat hemoglobin were purchased from Sigma (St. Louis, Mo, USA); LY 83583 (6-phenylamino-5,8-quinolinedione) was from Research Biochemicals International (Natick, Ma, USA); d$(CH_2)_5$Tyr(Me)-Orn^8-vasotocin was from Peninsula Laboratories (St. Helens/Mersey-side, UK). *S*-Nitroso-*N*-acetyl-penicillamine was prepared from *N*-acetyl-penicillamine as previously described (Field et al., 1978). Since commercial hemoglobins are usually a mixture of reduced and oxidized hemoglobins with different affinities for nitric oxide, rat hemoglobin was reduced with sodium dithionite, dialyzed against distilled water, divided in aliquots and stored at −20°C, as previously described (Martin et al., 1985).

2.3. I.c.v. and paraventricular nucleus injections

Stainless-steel guide cannulas (22 gauge, 0.71 mm) aimed unilaterally at the paraventricular nucleus of the hypothalamus were stereotaxically implanted (David Kopf Instruments, USA) under chloral hydrate anaesthesia 5 days before the experiments (coordinates: 0.2 mm anterior to bregma, 0.4 mm lateral to midline and 2.0 mm ventral to dura) (Pellegrino and Cushman, 1971). Animals were given 5 days to recover from surgery; each rat was used only once. The paraventricular nucleus-aimed guide cannula was used for either i.c.v. or paraventricular nucleus injections. In particular, for i.c.v. injections all substances were injected in a volume of 10 μl of saline in 1 min, except *S*-nitroso-*N*-acetyl-penicillamine which was dissolved in 95% ethanol and LY 83583 which was dissolved in methanol, via an internal cannula (28 gauge, 0.355 mm) that extended only 1 mm below the tip of the guide cannula in order to reach the ventricular space (see Pellegrino and Cushman, 1971). The internal cannula was connected by polypropylene tubing to a 10-μl Hamilton syringe driven by a micrometric screw. Both syringe and polypropylene tubing were protected from light with aluminium foil wrapped around them when nitric oxide donors were used. After injection the tip of the

cannula was left in the injection site for 30 s to allow the spread of the injected solution. For paraventricular nucleus injections substances were injected in a volume of only 0.3 μl of saline (95% ethanol for *S*-nitroso-*N*-acetyl-penicillamine and methanol for LY 83583) in 2 min via an internal cannula that extended 5.3 mm below the tip of the guide cannula in order to reach the paraventricular nucleus (see Pellegrino and Cushman, 1971). In this case the internal cannula was connected by polypropylene tubing to a 10-μl Hamilton syringe driven by a Stoelting microinfusion pump as already described (see Melis et al., 1992). As for i.c.v. injection, nitric oxide donors were protected from light with aluminium foil wrapped around the syringe and polypropylene tubing. After microinjection, the tip of the cannula was left in the injection site for 30 s in order to allow the spread of the injected solution.

2.4. Behavioral studies

Sodium nitroprusside, hydroxylamine, *S*-nitroso-*N*-acetyl-penicillamine or 8-Br-cGMP was given i.c.v. or in the paraventricular nucleus; isoamyl nitrite was given only i.c.v. Shortly after treatment, the animals were placed individually into Plexiglas cages (30 × 30 × 30 cm) and observed for 60 min, during which penile erection and yawning episodes were counted by an observer who was not aware of the treatments that had been performed in order to eliminate subjective evaluations. In those experiments in which methylene blue, LY 83583, hemoglobin or $d(CH_2)_5$-Tyr(Me)-Orn^8-vasotocin was used, the compounds were given i.c.v. or in the paraventricular nucleus 15 min before the nitric oxide donor. When i.c.v. injections were performed, the rats were killed by decapitation, brains were removed and visually inspected to ascertain the correct position of the cannula tip in the lateral ventricle. When substances were microinjected in the paraventricular nucleus, the rats were killed by decapitation and the brains were immediately removed and stored in saline containing 2% formaldehyde for 12–14 days. To localize the injection site, 50 μm transverse brain sections were prepared by means of a freezing microtome, stained with Neutral Red and inspected under a phase contrast microscope. The injection site was localized by following the internal cannula tract through a series of brain sections (see Fig. 1). Only those animals found to have the cannula tip positioned correctly in the lateral ventricles or in the PVN were considered for the statistical evaluation of the results.

2.5. Statistics

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

Fig. 1. 50 μm Neutral Red-stained transverse brain section from a sodium nitroprusside-responsive male rat, showing the tip of the cannula into the paraventricular nucleus of the hypothalamus (PVN); V = third ventricle, LH = lateral hypothalamus.

3. Results

3.1. Effect of nitric oxide donors injected i.c.v. on penile erection and yawning: dose-response curves

Fig. 2 shows the effect of sodium nitroprusside, hydroxylamine, isoamyl nitrite and *S*-nitroso-*N*-acetyl-

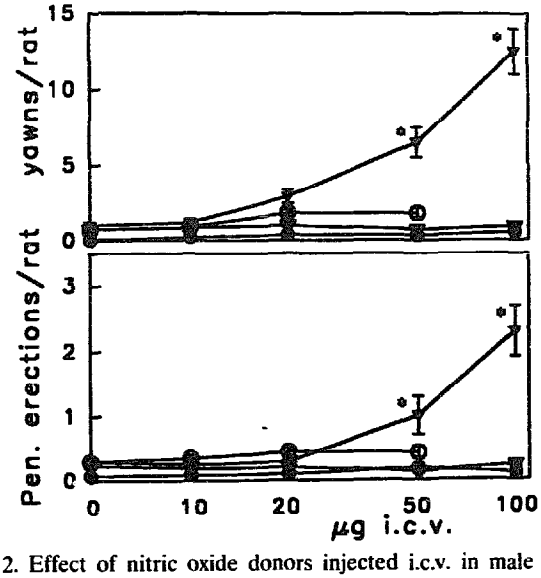

Fig. 2. Effect of nitric oxide donors injected i.c.v. in male rats on penile erection and yawning: dose-response curves. Sodium nitroprusside (○), hydroxylamine (●) and isoamyl nitrite (▼) were dissolved in saline and *S*-nitroso-*N*-acetyl-penicillamine (▽) in 95% ethanol, and all were injected i.c.v. in a volume of 10 μl. Controls received the same volume of vehicle i.c.v. (saline or 95% ethanol). After treatment, the rats were placed individually in Plexiglas cages and observed for 60 min, during which penile erection and yawning episodes were counted. Each value is the mean ± S.E.M. for ten rats. * $P < 0.01$ with respect to vehicle-treated rats (Duncan's multiple range test).

penicillamine given i.c.v. on spontaneous penile erection and yawning in male rats. Of the above compounds, only isoamyl nitrite (10–100 μg) induced penile erection and yawning dose dependently; the behavioral response was significant at 50 μg. In contrast, sodium nitroprusside (10–50 μg), hydroxylamine (10–100 μg) and *S*-nitroso-*N*-acetyl-penicillamine (10–100 μg) did not induce penile erection or yawning. However, behavioral changes that could have masked the above responses were observed at doses higher than 20 μg. In particular, sodium nitroprusside (20–50 μg) induced dose-dependent hypermotility, sniffing, rearing, convulsions often followed by death within 2–3 h after the treatment, while hydroxylamine (50–100 μg) induced immediately after treatment hyperactivity and convulsions that were rarely lethal, but which lasted for 10–20 min after which the rats looked normal. *S*-Nitroso-*N*-acetyl-penicillamine (50–100 μg) induced a dose-dependent hypermotility, sniffing and rearing that lasted for 40 min after treatment, but not convulsions or death (not shown).

3.2. Effect of nitric oxide donors injected into the paraventricular nucleus on penile erection and yawning: dose-response curves

Different results from those found after i.c.v. injection were obtained when the nitric oxide donors were

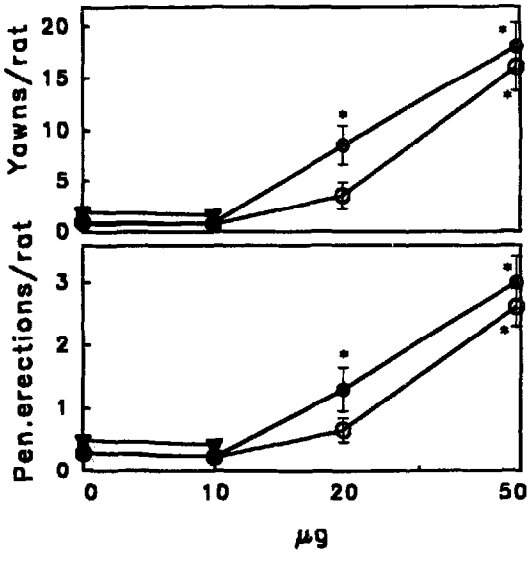

Fig. 3. Effect of nitric oxide donors injected into the paraventricular nucleus of male rats on penile erection and yawning: dose-response curves. Sodium nitroprusside (○) and hydroxylamine (●) were dissolved in saline and *S*-nitroso-*N*-acetyl-penicillamine (▼) in 95% ethanol, and all were injected monolaterally into the paraventricular nucleus in a volume of 0.3 μl, as described in Materials and methods. Controls received the same volume of vehicle in the paraventricular nucleus (saline or 95% ethanol). After treatment, the rats were placed individually in Plexiglas cages and observed for 60 min, during which penile erection and yawning episodes were counted. Each value is the mean ± S.E.M. for ten rats. * $P < 0.01$ with respect to vehicle-treated rats (Duncan's multiple range test).

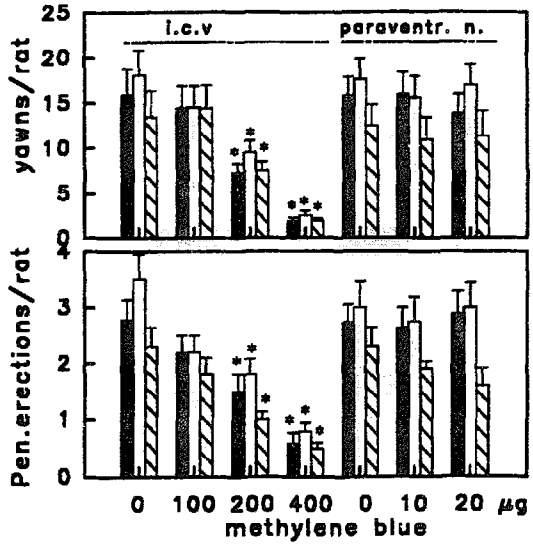

Fig. 4. Effect of methylene blue given i.c.v. or in the paraventricular nucleus on penile erection and yawning induced by nitric oxide donors. Methylene blue was given 15 min before sodium nitroprusside (50 μg in the paraventricular nucleus) (filled bars), or hydroxylamine (50 μg in the paraventricular nucleus) (empty bars) or isoamyl nitrite (100 μg i.c.v.) (hatched bars). After treatment, the rats were placed individually in Plexiglas cages and observed for 60 min, during which penile erection and yawning episodes were counted. Each value is the mean ± S.E.M. for eight rats. * $P < 0.01$ with respect to i.c.v. vehicle-pretreated rats (Duncan's multiple range test).

injected in the paraventricular nucleus. As shown in Fig. 3, sodium nitroprusside (10–50 μg) and hydroxylamine (10–50 μg) induced penile erection and yawning in a dose-dependent manner, although with a different potency. The most effective was hydroxylamine, which induced a significant effect already at the dose of 20 μg, while sodium nitroprusside was effective at the dose of 50 μg. In these experiments *S*-nitroso-*N*-acetyl-penicillamine was injected only at the dose of 10 μg due to its poor solubility. It was found to be ineffective, while isoamyl nitrite was not injected at all because of its insolubility and incompatibility with the other solvents (e.g. ethanol) and the impossibility of obtaining stable saline emulsions. The nitric oxide donors injected in the paraventricular nucleus did not induce any gross behavioral change nor the dramatic behavioral effects observed after their i.c.v. administration (i.e. hypermotility, sniffing, rearing or convulsion). However, after the higher dose of sodium nitroprusside in the paraventricular nucleus about 50% of the rats died within 12 h after the injection.

3.3. Nitric oxide donor-induced penile erection and yawning: effect of drugs that interfere with nitric oxide action injected i.c.v. or in the paraventricular nucleus

As shown in Fig. 4, methylene blue prevented dose dependently the penile erection and yawning induced by sodium nitroprusside (50 μg in the paraventricular

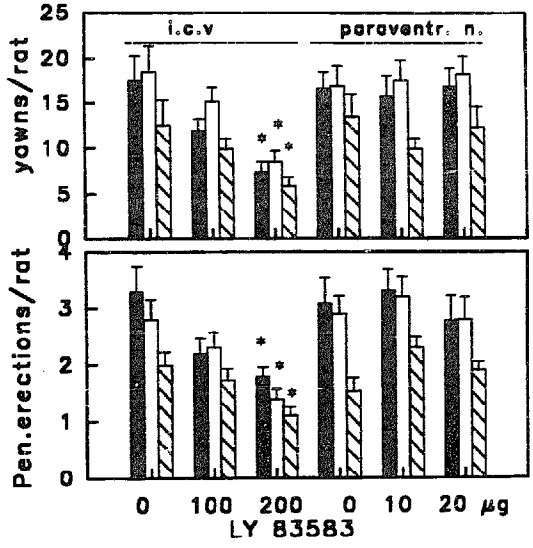

Fig. 5. Effect of LY 83583 given i.c.v. or in the paraventricular nucleus on penile erection and yawning induced by nitric oxide donors. LY 83583 was given 15 min before sodium nitroprusside (50 μg in the paraventricular nucleus) (filled bars), or hydroxylamine (50 μg in the paraventricular nucleus) (empty bars) or isoamyl nitrite (100 μg i.c.v.) (hatched bars). After treatment, the rats were placed individually in Plexiglas cages and observed for 60 min, during which penile erection and yawning episodes were counted. Each value is the mean ± S.E.M. for eight rats. * $P < 0.01$ with respect to i.c.v. vehicle-pretreated rats (Duncan's multiple range test).

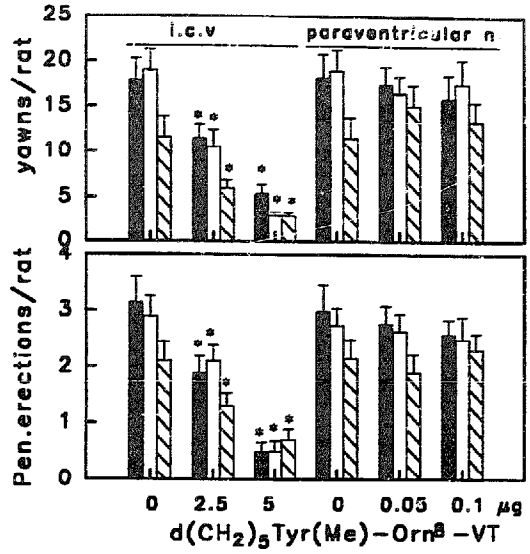

Fig. 7. Effect of $d(CH_2)_5Tyr(Me)$-Orn^8-vasotocin given i.c.v. or in the paraventricular nucleus on penile erection and yawning induced by nitric oxide donors. $d(CH_2)_5Tyr(Me)$-Orn^8-vasotocin was given 15 min before sodium nitroprusside (50 μg in the paraventricular nucleus) (filled bars), or hydroxylamine (50 μg in the paraventricular nucleus) (empty bars) or isoamyl nitrite (100 μg i.c.v.) (hatched bars). After treatment, the rats were placed individually in Plexiglas cages and observed for 60 min, during which penile erection and yawning episodes were counted. Each value is the mean ± S.E.M. for eight rats. * $P < 0.01$ with respect to i.c.v. vehicle-pretreated rats (Duncan's multiple range test).

nucleus), or hydroxylamine (50 μg in the paraventricular nucleus) or isoamyl nitrite (100 μg i.c.v.) when given i.c.v. (100–400 μg) but not when given into the paraventricular nucleus (10–20 μg) 15 min before the nitric oxide donor. Similar results were found with LY 83583, which prevented penile erection and yawning induced by the nitric oxide donors when given i.c.v. (100–200 μg), but not when injected in the paraventricular nucleus (10–20 μg) (Fig. 5). In contrast, hemoglobin was ineffective in preventing the above behavioral responses when injected i.c.v. (100–400 μg) or in the paraventricular nucleus (10–20 μg) (Fig. 6).

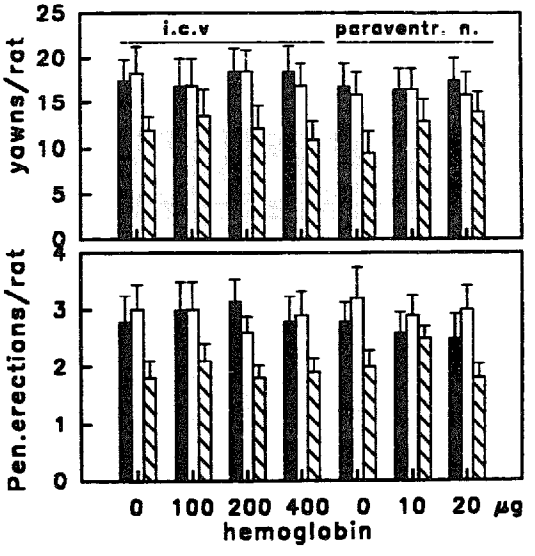

Fig. 6. Effect of hemoglobin given i.c.v. or in the paraventricular nucleus on penile erection and yawning induced by nitric oxide donors. Hemoglobin was given 15 min before sodium nitroprusside (50 μg in the paraventricular nucleus) (filled bars), or hydroxylamine (50 μg in the paraventricular nucleus) (empty bars) or isoamyl nitrite (100 μg i.c.v.) (hatched bars). After treatment, the rats were placed individually in Plexiglas cages and observed for 60 min, during which penile erection and yawning episodes were counted. Each value is the mean ± S.E.M. for eight rats. * $P < 0.01$ with respect to i.c.v. vehicle-pretreated rats (Duncan's multiple range test).

3.4. Nitric oxide donor-induced penile erection and yawning: effect of $d(CH_2)_5Tyr(Me)$-Orn^8-vasotocin given i.c.v. or in the paraventricular nucleus

As shown in Fig. 7, $d(CH_2)_5Tyr(Me)$-Orn^8-vasotocin prevented the penile erection and yawning induced by sodium nitroprusside (50 μg in the paraventricular nucleus), hydroxylamine (50 μg in the paraventricular nucleus) or isoamyl nitrite (100 μg i.c.v.) when given i.c.v. (2.5–5 μg) but not when injected into the paraventricular nucleus (0.05 –0.1 μg) 15 min before the nitric oxide donor.

3.5. Effect of 8-Br-cGMP injected i.c.v. or in the paraventricular nucleus on penile erection and yawning

As shown in Table 1, unlike the nitric oxide donors, 8-Br-cGMP injected either i.c.v. (10–400 μg) or in the

Table 1
Effect of 8-Br-cGMP injected i.c.v. or in the paraventricular nucleus on spontaneous penile erection and yawning in male rats: comparison with nitric oxide donors

Treatment	Dose	Penile erections/rat	Yawns/rat
Saline	10 μl i.c.v.	0.2 ± 0.03	1.3 ± 0.2
8-Br-cGMP	10 μg i.c.v	0.4 ± 0.03	1.3 ± 0.3
8-Br-cGMP	50 μg i.c.v	0.2 ± 0.05	0.9 ± 0.3
8-Br-cGMP	100 μg i.c.v	0.4 ± 0.05	1.5 ± 0.3
8-Br-cGMP	200 μg i.c.v.	0.6 ± 0.1	1.4 ± 0.4
8-Br-cGMP	400 μg i.c.v.	0.5 ± 0.09	1.6 ± 0.3
Isoamyl nitrite	100 μg i.c.v.	2.8 ± 0.8 [a]	12.7 ± 0.6 [a]
Saline	0.3 μl paraventr. n.	0.6 ± 0.2	1.8 ± 0.4
8-Br-cGMP	10 μg paraventr. n.	0.8 ± 0.3	2.0 ± 0.4
8-Br-cGMP	20 μg paraventr. n.	0.8 ± 0.4	2.2 ± 0.3
8-Br-cGMP	50 μg paraventr. n.	0.5 ± 0.2	2.0 ± 0.3
Sodium nitroprusside	50 μg paraventr. n.	3.0 ± 0.6 [a]	16.3 ± 2.5 [a]
Hydroxylamine	50 μg paraventr. n.	3.6 ± 0.5 [a]	18.5 ± 2.0 [a]

After i.c.v. or paraventricular injections, the rats were placed individually into Plexiglas cages and observed for 60 min during which the number of penile erection and yawning episodes were counted. Each value is the mean ± S.E.M. for 10 rats per group. [a] $P < 0.01$ with respect to the corresponding saline-treated rats (Duncan's multiple range test).

paraventricular nucleus (10–50 μg) did not induce penile erection or yawning. However, like nitric oxide donors, when injected i.c.v. at doses higher than 100 μg the compound induced a dose-dependent hypermotility, sniffing and rearing that lasted for 40 min after treatment, but not convulsions, which could have masked penile erection and yawning (not shown). In contrast, no gross behavioral change was observed when 8-Br-cGMP was injected in the paraventricular nucleus.

4. Discussion

The present results show that the putative nitric oxide donors sodium nitroprusside, hydroxylamine and isoamyl nitrite induce penile erection and yawning when injected in the central nervous system, in particular in the paraventricular nucleus of the hypothalamus. In fact, these behavioral responses were not seen when nitric oxide donors, with the exception of isoamyl nitrite, were injected into the lateral ventricles, because these responses might have been masked by the often dramatic and different behavioral effects that were probably due to the different affinity and/or metabolic biotransformation of these agents in specific brain tissues (see Gross et al., 1994). In fact, in agreement with previous studies, although both *S*-nitroso-*N*-acetyl-penicillamine and sodium nitroprusside injected i.c.v. induced hypermotility, the latter induced also convulsions followed by death, probably due to the formation of highly toxic cyanide ions (Gross et al., 1994). It is likely that the formation of cyanide ions that diffuse into the brain is responsible also for the death of about 50% of the rats injected in the paraventricular nucleus with the highest dose of sodium nitroprusside 12 h after treatment. Likewise, hydroxylamine was ineffective when injected i.c.v. because of its potent convulsant effect but was very active when injected locally in the paraventricular nucleus. The ineffectiveness of *S*-nitroso-*N*-acetyl-penicillamine, in spite of its ability to release nitric oxide spontaneously even more effectively than sodium nitroprusside and hydroxylamine (Southam and Garthwaite, 1991), is difficult to explain. However, one possible explanation is that the dose injected in the paraventricular nucleus (10 μg) was too low to induce the behavioral responses. In fact higher doses were not injected because this compound was poorly soluble even in 95% ethanol and effective nitric oxide donors were active only at doses higher than 20 μg.

The present results confirm and extend previous findings showing that nitroglycerin, another nitric oxide donor, induces penile erection and yawning when injected i.c.v. or in the paraventricular nucleus (Argiolas, 1994; Melis et al., 1995a) and provide further evidence that paraventricular nitric oxide plays a key role in the control of penile erection and yawning. Indeed, (1) nitric oxide-synthase inhibitors prevent apomorphine-, oxytocin- and NMDA-induced penile erection and yawning when injected in the paraventricular nucleus (Melis et al., 1994b,c); (2) the prevention by nitric oxide-synthase inhibitors of apomorphine-, oxytocin- and NMDA-induced penile erection and yawning is reversed by L-arginine (Melis and Argiolas, in preparation); (3) the paraventricular nucleus is one of the brain areas richest in nitric oxide-synthase (Vincent and Kimura, 1992; Southam and Garthwaite, 1993); and (4) nitric oxide-synthase is localized in paraventricular oxytocinergic neurons (Bredt et al., 1990; Sanchez et al., 1994). It is conceivable that the nitric oxide donors induce these behavioral responses by producing

nitric oxide in the paraventricular nucleus, which would in turn activate central oxytocinergic transmission, since the penile erection and yawning induced by sodium nitroprusside, hydroxylamine and isoamyl nitrite as well as nitroglycerin (Melis et al., 1995a) were prevented by the i.c.v. injection of d(CH_2)$_5$Tyr(Me)-Orn^8-vasotocin, a potent oxytocin receptor antagonist (Bankowski et al., 1980). However, since d(CH_2)$_5$Tyr(Me)-Orn^8-vasotocin was ineffective when given in the paraventricular nucleus, it is likely that drug-derived nitric oxide, as proposed for endogenous nitric oxide (Argiolas, 1994; Melis et al., 1994b,c), activates oxytocinergic neurons originating in the paraventricular nucleus and projecting to extra-hypothalamic areas. The hippocampus or the ventral medulla/spinal cord might be among these areas, since both receive an oxytocinergic innervation and are involved in the expression of these behavioral responses to oxytocin (see Melis et al., 1992; Stancampiano et al., 1994).

The molecular mechanism by which endogenous or drug-derived nitric oxide in the paraventricular nucleus activates oxytocinergic neurons to induce penile erection and yawning is unknown. One possibility is that nitric oxide activates guanylate cyclase, one of the most common targets of this modulator in several tissues including the brain (see Schuman and Madison, 1994 and enclosed references), which would in turn increase the concentration of the second messenger cGMP. Accordingly, methylene blue and LY 83583, two guanylate cyclase inhibitors (Murad et al., 1978; Gruetter et al., 1981; Mülsch et al., 1988), given into the lateral ventricles prevent nitric oxide donor-induced penile erection and yawning. However, the involvement of guanylate cyclase and cGMP, especially at the level of the paraventricular nucleus, in these behavioral responses must be considered with caution for at least three reasons. First, methylene blue and LY 83583 did not prevent nitric oxide donor-induced penile erection and yawning when injected into the paraventricular nucleus; second, hemoglobin, a potent nitric oxide scavenger (Murad et al., 1978; Gruetter et al., 1981), was ineffective in preventing nitric oxide donor-induced responses when injected into the paraventricular nucleus; and third, perhaps most important, 8-Br-cGMP, a stable cGMP analog that can mimic the effect of endogenous cGMP produced by nitric oxide activation of guanylate cyclase, was unable to induce penile erection and yawning when injected into the paraventricular nucleus. Although the inability of hemoglobin to prevent nitric oxide donor-induced responses when injected directly in the paraventricular nucleus might be explained by the inability of this compound to cross cellular membranes because of its high molecular weight (Gruetter et al., 1981), all together the above findings suggest that these substances induce penile erection and yawning after their injection in the paraventricular nucleus by a mechanism not related to the stimulation of guanylate cyclase, that is by a cGMP-independent mechanism. Accordingly, if one assumes that nitric oxide donor-induced responses are mediated by nitric oxide produced in target neurons, nitric oxide might interact with numerous other enzymes that, like guanylate cyclase, bind metal ions such as iron, as described for instance in fibroblasts (Garg and Hassid, 1991) (for a review on other possible targets of nitric oxide see Schuman and Madison, 1994).

Whatever mechanism nitric oxide activates in the paraventricular nucleus to induce penile erection and yawning, the ineffectiveness of hemoglobin, which because of its high molecular weight is unable to cross cellular membranes (Gruetter et al., 1981), to prevent nitric oxide donor-induced responses suggests also that nitric oxide acts intracellularly, possibly in the same neurons in which it is formed, to induce penile erection and yawning. This implies that nitric oxide released extracellularly in the paraventricular nucleus is not involved in the control of these behavioral responses. This hypothesis is complicated by the finding that methylene blue or LY 83583 given i.c.v. prevented nitric oxide donor-induced responses, which favours a neurotransmitter role of nitric oxide in brain areas distant from the paraventricular nucleus rather than a local messenger role in the paraventricular nucleus. A possible unifying explanation for such discrepancy is that nitric oxide activates those oxytocinergic neurons where it is formed, releasing in turn oxytocin which activates directly or indirectly a methylene blue- or LY 83583-sensitive guanylate cyclase mechanism in extra-hypothalamic brain areas. This hypothesis is supported by the above discussed ability of d(CH_2)$_5$Tyr(Me)-Orn^8-vasotocin given i.c.v. to prevent nitric oxide donor-induced penile erection and yawning. Unfortunately, the present results do not provide definitive evidence for a role of guanylate cyclase in extra-hypothalamic brain areas in the control of penile erection and yawning. In fact, methylene blue or LY 83583 given i.c.v. might prevent nitric oxide donor-induced penile erection and yawning by acting with a mechanism not related to the inhibition of guanylate cyclase (see Mülsch et al., 1988; Marczin et al., 1992; Mayer et al., 1993), and secondly 8-Br-cGMP injected i.c.v. induced a marked hyperactivity that masked the above responses and resembled that induced by nitric oxide donors, which is partially reversed by methylene blue (Gross et al., 1994).

The interpretation given above of the present results is based on two main assumptions. The first is the existence of central oxytocinergic projections originating in the paraventricular nucleus and projecting to extra-hypothalamic brain areas that mediate penile erection and yawning. These pathways are activated by several neurotransmitters, such as dopamine, glutamic

acid and oxytocin itself, and this activation is secondary to the activation of nitric oxide synthase in the cell bodies of paraventricular oxytocinergic neurons. The second assumption is that nitric oxide donors act exclusively by increasing the nitric oxide concentration in the injection site, in particular in the cell bodies of oxytocinergic neurons, to cause penile erection and yawning. Although the possibility that nitric oxide donors induce the above responses by other mechanisms cannot be completely ruled out, this assumption is strongly supported by the ability of several nitric oxide donors to induce similar and dose-dependent responses apparently mediated by the same mechanisms.

In conclusion, nitric oxide donors induce penile erection and yawning when injected into the paraventricular nucleus by activating oxytocinergic transmission. These responses are apparently not mediated by the activation of guanylate cyclase in this hypothalamic nucleus, although a role of this enzyme and thus of cGMP in extra-hypothalamic brain areas cannot be ruled out. Interestingly nitric oxide donors, e.g. nitroglycerin and sodium nitroprusside, have been also found to induce penile erection in impotent men when used as topical ointments or after intracavernosal administration (see Nunez and Anderson, 1993 and enclosed references), and isoamyl nitrite has been known for its aphrodisiac effect for many years (Everett, 1975). Our results show that nitric oxide plays a key role in the control of penile erection not only at a penile level (Burnett et al., 1992; Rajfer et al., 1992), but also in the central nervous system.

Acknowledgements

This work was partially supported by a MURST grant to A.A.

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