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Prevention by N^G-Nitro-L-Arginine Methyl Ester of Apomorphine- and Oxytocin-Induced Penile Erection and Yawning: Site of Action in the Brain

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MELIS, M. R., R. STANCAMPIANO AND A. ARGIOLAS. *Prevention by N^G-nitro-L-arginine methyl ester of apomorphine- and oxytocin-induced penile erection and yawning: Site of action in the brain.* PHARMACOL BIOCHEM BEHAV **48**(3) 799–804, 1994.—The effect of N^G-nitro-L-arginine methyl ester (NAME), a potent inhibitor of nitric oxide (NO) synthase, injected into different brain areas on penile erection and yawning induced by apomorphine or oxytocin was studied in male rats. The compound was found to be able to prevent the above behavioral responses dose dependently when injected into the paraventricular nucleus of the hypothalamus (PVN), but not in the caudate nucleus, medial septum, preoptic area, and the CA1 field of the hippocampus. When injected in the PVN, 5 μg of NAME induced a 30% reduction of apomorphine and oxytocin responses, while 20 μg induced an almost complete reduction. The effect of NAME seems to be related to the inhibition of guanylate cyclase secondary to the prevention of NO formation, because a dose-dependent reduction of apomorphine and oxytocin responses was obtained also with the inhibitor of guanylate cyclase methylene blue injected intracerebroventricularly (100–400 μg ICV), but not into the PVN. The results provide further support for a neurotransmitter role of central NO in the control of penile erection and yawning.

Penile erection Yawning Nitric oxide Apomorphine Oxytocin
Paraventricular nucleus of the hypothalamus Rat

PENILE erection and yawning are two different behavioral patterns that often occur concomitantly in physiological and experimental conditions (14). Oxytocin and apomorphine are among the most potent substances able to induce penile erection and yawning discovered so far (1,19,20) and a neuronal dopamine-oxytocin link in the paraventricular nucleus of the hypothalamus (PVN) seems to be involved in the induction of these behavioral responses [see (21,22)]. As far as the molecular mechanism by means of which apomorphine and oxytocin induce these behavioral effects is concerned, calcium and pertussis toxin-sensitive G proteins seem to be involved, because nanogram amounts of the potent N-type calcium channel blocker omega-conotoxin-GVIA (2) or of pertussis toxin (30) prevent the above symptomatology when injected in the PVN.

Recently we found that both peripheral and central administration of inhibitors of nitric oxide (NO) synthase (28), the enzyme responsible for the formation of NO, prevent apomorphine- and oxytocin-induced penile erection and yawning (23), suggesting that this novel discovered transduction messenger (4,5,12,16,29) is also involved in the control of these responses induced by apomorphine and oxytocin. To further characterize the role of NO in the control of these responses, we studied the effect of the microinjection of N^G-nitro-L-arginine methyl ester (NAME), one of the most potent inhibitors of NO synthase available so far (8), in different brain areas on apomorphine- and oxytocin-induced penile erection and yawning. Moreover, because NO is supposed to act by stimulating guanylate cyclase to produce cyclic guanosine 3′,5′ monophosphate (cGMP) (4,8,12,15–17,25), the effect of the central microinjection of methylene blue, an inhibitor of guanylate

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cyclase (25) on apomorphine- and oxytocin-induced penile erection and yawning was also studied.

METHOD

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%, with water and standard laboratory food ad lib.

Drugs

N^G-nitro-L-arginine methyl ester (NAME), methylene blue, and apomorphine-HCl were purchased from Sigma (St. Louis, MO); oxytocin from Peninsula Laboratories Inc. (UK).

Chronic Guide Cannulae Implantation

Stainless steel guide cannulae (22 gauge) aimed at one lateral ventricle (ICV) or bilaterally at the hippocampus (CA1 field), preoptic area, and caudate nucleus or unilaterally at the PVN and medial septum were stereotaxically implanted under chloral hydrate anesthesia 5 days before the experiments (coordinates: preoptic area 2.0 mm anterior to bregma, 1.5 mm lateral to midline, and 2 mm ventral to dura; hippocampal CA1 field 3.0 mm posterior to bregma, 1.8 mm lateral to midline, and 2.0 mm ventral to dura; paraventricular nucleus (PVN) 0.2 mm anterior to bregma, 0.4 mm lateral to midline, and 2.0 mm ventral to dura; medial septum 2.0 mm anterior to bregma, along the midline, and 2.0 mm ventral to dura; caudate nucleus 2.6 mm anterior to bregma, 3.0 mm lateral to midline, and 2.0 mm ventral to dura) (27). Animals were given 5 days to recover from surgery; each rat was used only once. Saline (5 μl in 15 s), NAME, methylene blue, and oxytocin dissolved in saline were injected ICV via an internal cannula (28 gauge) that extended 2 mm below the tip of the guide cannula and connected by polyethylene tubing to a 10 μl Hamilton syringe driven by a micrometric screw. For PVN, preoptic area, hippocampal, medial septal, and striatal microinjections, substances were dissolved in saline (0.3 μl in 2 min) and injected by means of an internal cannula (28 gauge) that extended 5.3 mm for the PVN, 6 mm for the preoptic area, 1 mm for the hippocampus, 4 mm for the medial septum, and 3 mm for the caudate nucleus, respectively, below the tip of the guide cannula and connected to a 10 μl Hamilton syringe driven by a Stoelting microinfusion pump. After microinjections, the tip of the cannula was left in the injection site for 30 s to allow the spread of the injected solution.

Systemic Treatments

Apomorphine-HCl was dissolved in saline and injected subcutaneously (SC) in a volume of 0.2 ml/200 g body weight. Controls received the same volume of SC saline.

Behavioral Studies

NAME and methylene blue were microinjected 15 min before apomorphine or oxytocin. Shortly after oxytocin or apomorphine, animals were placed individually into Plexiglas cages (30 × 30 × 30 cm) and observed for 60 min after oxytocin or apomorphine, during which penile erection and yawning episodes were counted. At the end of experiments, animals were killed by decapitation, brains were removed, and visually inspected to ascertain the correct position of the cannula tip into the lateral ventricle. Only those animals that were found to have the internal cannula tip positioned correctly in the lateral ventricle were considered for statistical analysis of the results.

Histology

In experiments in which striatal, hippocampal, preoptic area, PVN, or medial septum microinjections were performed, at the end of the experiments animals were killed by decapitation and brains removed and stored in saline containing 2% formaldehyde for 12–15 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 on a phase contrast microscope. The injection site was localized by following the internal cannula tract through a series of brain sections. Only those animals found to have the internal cannula tip positioned correctly in the PVN, CA1 field of the hippocampus, preoptic area, medial septum, or caudate nucleus were considered for statistical analysis of the results.

TABLE 1

EFFECT OF NAME INJECTED INTO DIFFERENT BRAIN AREAS ON SPONTANEOUS PENILE ERECTION AND YAWNING

	Microinjections			
	Saline		NAME	
Brain area	Penile Erections/Rat	Yawns/Rat	Penile Erections/Rat	Yawns/Rat
Caudate n.	0.4 ± 0.05	1.2 ± 0.4	0.2 ± 0.01	0.4 ± 0.05
Med. Septum	0.3 ± 0.05	1.1 ± 0.3	0.4 ± 0.03	1.3 ± 0.30
Hippocampus (CA1 field)	0.5 ± 0.08	1.5 ± 0.4	0.3 ± 0.04	1.5 ± 0.50
PVN	0.6 ± 0.06	1.3 ± 0.2	0.5 ± 0.02	1.2 ± 0.30
Preoptic area	0.4 ± 0.03	1.0 ± 0.2	0.4 ± 0.03	1.3 ± 0.40

Saline (0.3 μl) alone or containing NAME was injected bilaterally in the caudate nucleus, the CA1 field of the hippocampus, or the preoptic area (10 μg in each side), and unilaterally in the PVN and centrally in the medial septum (20 μg in each area). After treatment, rats were placed individually into Plexiglas cages and observed for 60 min, in order to count penile erections and yawns. Values are the mean ± SEM of nine rats per group.

TABLE 2

EFFECT OF NAME INJECTED INTO DIFFERENT BRAIN AREAS ON APOMORPHINE-INDUCED PENILE ERECTION AND YAWNING

	Microinjections			
	Saline		NAME	
Brain area	Penile Erections/Rat	Yawns/Rat	Penile Erections/Rat	Yawns/Rat
Caudate n.	2.8 ± 0.4	17.5 ± 2.0	3.0 ± 0.4	18.5 ± 2.1
Med. Septum	2.3 ± 0.5	17.1 ± 1.3	2.5 ± 0.3	19.1 ± 2.0
Hippocampus (CA1 field)	2.5 ± 0.4	18.5 ± 1.4	2.3 ± 0.4	18.5 ± 1.8
PVN	2.6 ± 0.3	17.3 ± 2.2	0.5 ± 0.02*	4.7 ± 0.5*
Preoptic area	2.4 ± 0.3	18.5 ± 2.5	2.4 ± 0.3	18.8 ± 2.3

Saline (0.3 μl) alone or containing NAME was injected bilaterally in the caudate nucleus, the CA1 field of the hippocampus, or the preoptic area (10 μg in each side), and unilaterally in the PVN and centrally in the medial septum (20 μg in each area) 15 min before apomorphine (80 μg/kg SC). After treatment, rats were placed individually into Plexiglas cages and observed for 60 min, in order to count penile erections and yawns. Values are the mean ± SEM of 10 rats per group.

*$p < 0.01$ with respect to PVN saline-treated rats (Mann–Whitney test).

Statistics

Statistical evaluation of the results was performed by the Mann–Whitney test for comparison of differences between two groups, and by one-way ANOVA, followed by Dunnett's test for comparison of differences among multiple groups. A $p < 0.05$ was considered significant.

RESULTS

Table 1 shows that NAME injected bilaterally into the caudate nucleus, hippocampal CA1 field or the preoptic area (10 μg in each side), and unilaterally into the PVN (20 μg) or the medial septum (20 μg), did not induce, per se, any behavioral change. However, when injected into the PVN, but not in the other brain areas, NAME reduced penile erection and yawning induced by apomorphine (80 μg/kg SC) ($n = 10$, $U = 8$ and 0 for penile erection and yawning, respectively, $p < 0.01$) (Table 2) or oxytocin (30 ng ICV) ($n = 10$, $U = 7$ and 1 for penile erection and yawning, respectively, $p < 0.01$) (Table 3).

Histological analysis of brain sections from rats injected in the PVN and surrounding sites revealed that to reduce penile erection and yawning induced by apomorphine or oxytocin, NAME had to be injected in the PVN, because injections placed laterally, dorsally, ventrally, rostrally, or caudally to the nucleus were ineffective (Fig. 1).

As shown in Fig. 2, NAME injected in the PVN reduced apomorphine- and oxytocin-induced penile erection and yawning in a dose-dependent manner. A 30% reduction of apomorphine and oxytocin effects was already seen at the dose of 5 μg, while about 80% reduction was observed at the dose of 20 μg [$df = 47$, $F(3, 44) = 26.35$ and 20.43, and 526.39 and 448.48 for apomorphine- and oxytocin-induced penile erection and yawning, respectively, $p < 0.01$]. In contrast, no reduction of apomorphine and oxytocin-induced penile erection and yawning was observed when methylene blue

TABLE 3

EFFECT OF NAME INJECTED INTO DIFFERENT BRAIN AREAS ON OXYTOCIN-INDUCED PENILE ERECTION AND YAWNING

	Microinjections			
	Saline		NAME	
Brain area	Penile Erections/Rat	Yawns/Rat	Penile Erections/Rat	Yawns/Rat
Caudate n.	3.5 ± 0.5	17.8 ± 2.0	3.3 ± 0.4	18.5 ± 2.8
Med. Septum	3.3 ± 0.5	18.1 ± 1.3	2.7 ± 0.3	20.5 ± 3.0
Hippocampus (CA1 field)	3.5 ± 0.8	19.5 ± 2.4	2.8 ± 0.5	20.3 ± 2.8
PVN	3.6 ± 0.6	18.3 ± 1.2	0.6 ± 0.04*	4.1 ± 0.6*
Preoptic area	3.4 ± 0.6	17.5 ± 2.2	2.7 ± 0.4	20.4 ± 3.1

Saline (0.3 μl) alone or containing NAME was injected bilaterally in the caudate nucleus, the CA1 field of the hippocampus, or the preoptic area (10 μg in each side), and unilaterally in the PVN and centrally in the medial septum (20 μg in each area) 15 min before oxytocin (30 ng ICV). After treatment, rats were placed individually into Plexiglas cages and observed for 60 min, in order to count penile erections and yawns. Values are the mean ± SEM of 10 rats per group.

*$p < 0.01$ with respect to PVN saline-treated rats (Mann–Whitney test).

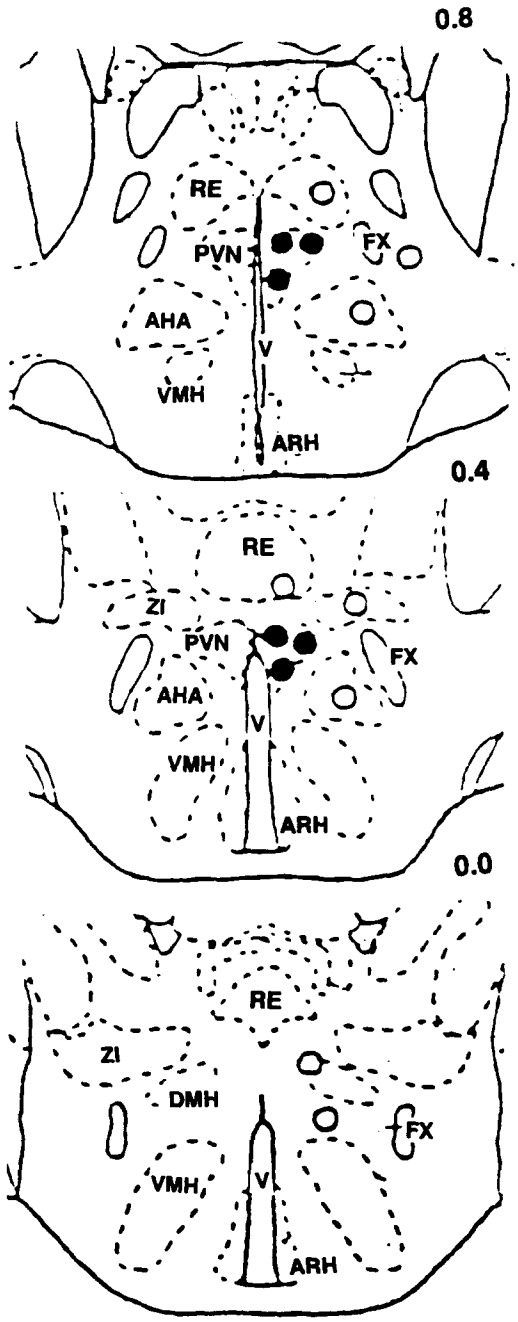

FIG. 1. Schematic representation of the injection sites in which NAME was found to be able and unable to reduce apomorphine- or oxytocin-induced penile erection and yawning, respectively. Chronic guide cannulae were unilaterally implanted 0.8, 0.4, or 0.0 mm anterior to bregma, with the lateral distance from sagittal suture between 0 and 2 mm (27). The tip of the internal cannula was lowered 6.5–7.5 mm from dura. Filled and empty circles represent the sites in which the drug dissolved in a volume of 0.3 μl was found to be active and inactive, respectively. Abbreviations: AHA = anterior hypothalamus; ARH = arcuate nucleus of the hypothalamus; DMH = dorsomedial nucleus of the hypothalamus; FX = fornix; PVN = paraventricular nucleus of the hypothalamus; RE = nucleus reuniens thalami; V = third ventricle; ZI = zona incerta.

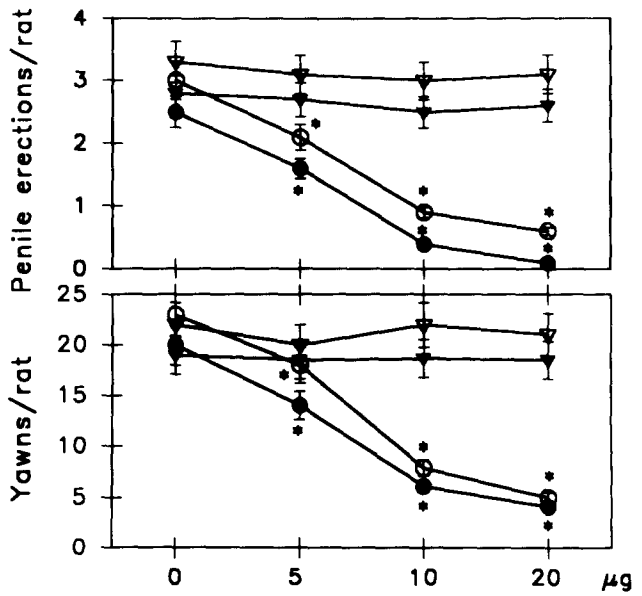

FIG. 2. Effect of NAME and methylene blue microinjected in the PVN on penile erection and yawning induced by apomorphine (filled symbols) or by oxytocin (empty symbols). NAME (circles) and methylene blue (triangles) were microinjected into the PVN 15 min before apomorphine (80 μg/kg SC) or oxytocin (30 ng ICV). After treatment, rats were placed individually into Plexiglas cages and observed for 60 min, to count penile erections and yawns. Values are the means ± SEM of 12 rats per group. *$p < 0.01$ with respect to PVN saline-treated rats (μg PVN NAME or methylene blue = 0) (one-way ANOVA followed by Dunnett's test).

(5–20 μg) was injected in the PVN instead of NAME [df = 47, $F(3, 44)$ = 0.86 and 0.96, and 1.92 and 1.53 for apomorphine- and oxytocin-induced penile erection and yawning, respectively, not significant at $p < 0.05$] (Fig. 2). In spite of its inefficacy when injected into the PVN, methylene blue-injected ICV was as effective as but less potent than ICV NAME in reducing these behavioral responses (Fig. 3). Indeed, while NAME was effective at doses between 30–150 μg [df = 55, $F(3, 52)$ = 47.5 and 27.13, and 927.77 and 470.69 for apomorphine- and oxytocin-induced penile erection and yawning, respectively, $p < 0.01$], methylene blue was active at doses between 100–400 μg [df = 55, $F(3, 52)$ = 45.98 and 49.39, and 946.46 and 792.02 for apomorphine- and oxytocin-induced penile erection and yawning, respectively, $p < 0.01$].

DISCUSSION

The present results show that the PVN is a brain site where NO synthase inhibitors act to antagonize penile erection and yawning induced by apomorphine or oxytocin. In agreement with this hypothesis, the PVN is one of the most rich brain areas containing NO synthase-immunostaining neurons (31). This confirms and extends previous results showing that central NO is involved in the control of these behavioral responses (23). In particular, the ability of NAME to prevent penile erection when injected in the PVN not only suggests that this novel neurotransmitter is involved in the control of this primary sexual function, but also provides further evidence for a major role of this hypothalamic nucleus in the control of sexual functions strictly related to the consummatory phase of

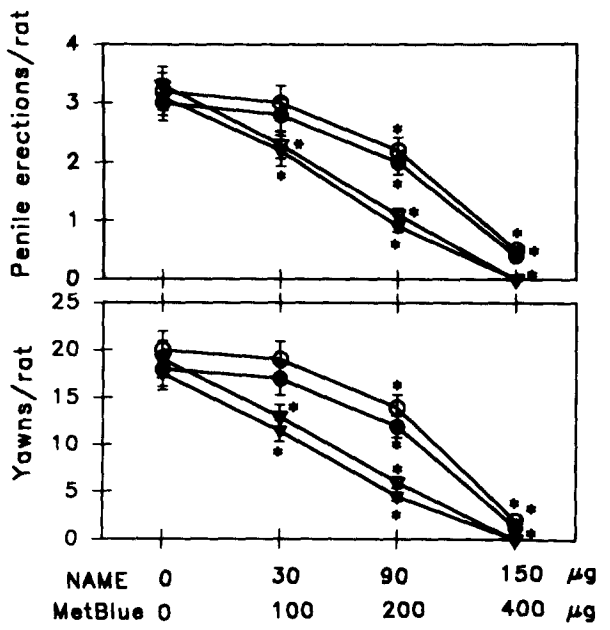

FIG. 3. Effect of ICV NAME and methylene blue on penile erection and yawning induced by apomorphine (filled symbols) or by oxytocin (empty symbols). NAME (circles) and methylene blue (triangles) were injected ICV 15 min before apomorphine (80 μg/kg SC) or oxytocin (30 ng ICV). After treatment, rats were placed individually into Plexiglas cages and observed for 60 min, to count penile erections and yawns. Values are the means ± SEM of 14 rats per group. *$p <$ 0.01 with respect to ICV saline-treated rats (μg ICV NAME or methylene blue = 0) (one-way ANOVA followed by Dunnett's test). Legends of the figures.

sexual behavior, i.e., penile erection, penile reflexes, and seminal emission (10,19,20).

The mechanism by which NAME acts in the PVN to prevent penile erection and yawning is unknown, and only some speculation is possible at present. If one assumes that apomorphine induces these responses by activating oxytocinergic neurons in the PVN (see introductory paragraphs), NAME might act by preventing the activation of oxytocinergic transmission. In this respect, it is pertinent to recall that apomorphine and oxytocin effects seem to be mediated by an increase in calcium ion influx (2). As already suggested for the NMDA receptor-coupled calcium channel (3), calcium ions might bind to calmodulin activating in turn NO synthase (4) to produce NO that activates guanylate cyclase to increase cGMP production (4,8,12,15-17,25). If this hypothesis were correct, NO would be considered either as a second messenger, like calcium ions, or a neurotransmitter itself that mediates penile erection and yawning induced by apomorphine and oxytocin. In agreement with the above hypothesis, oxytocinergic neurons, when activated by apomorphine or oxytocin itself [see (19)], should produce NO that might be released either by neuronal cell bodies and/or nerve endings to act as a neurotransmitter activating in turn the production of cGMP in target cells [for a review see (24)] or might act intracellularly as a second messenger. A neurotransmitter role of NO at sites distant from the PVN is favored by the results obtained with methylene blue, an inhibitor of guanylate cyclase in several tissues, i.e., platelets (7) and vascular smooth muscles (13). Indeed, methylene blue is unable to prevent penile erection and yawning induced by apomorphine and oxytocin when injected in the PVN, but is fully active when injected ICV. This implies that cGMP is involved in the effects of apomorphine and oxytocin in some yet undiscovered brain area. This explanation should be considered with caution, because it was recently reported that methylene blue can inhibit directly NO synthase rather than guanylate cyclase (18). However, the inability of methylene blue, unlike NAME, to prevent penile erection and yawning when injected in the PVN does not support such possibility in our experimental conditions.

On the other hand, the ineffectiveness of methylene blue injected in the PVN might indicate that NO released by oxytocinergic cell bodies in the PVN influences the expression of penile erection and yawning by acting with a mechanism not related to the activation of guanylate cyclase, that is, by a cGMP-independent mechanism. NO infact might interact with numerous other enzymes that, like guanylate cyclase, bind metal ions such as iron, as described for instance in fibroblasts (11). The identification of the brain area in which the inhibition of guanylate cyclase by methylene blue or other inhibitors prevents apomorphine and oxytocin responses will clarify this point.

The above interpretations are in line with the existence of a neuronal dopamine-oxytocin link that controls these behavioral responses, in particular, with previous findings showing that apomorphine effect on penile erection and yawning are prevented by oxytocin antagonists given ICV but not injected into the PVN [see (22)]. However, the present results do not rule out the possibility that NAME induces its effects by acting in the PVN at targets different from the cell bodies of parvocellular oxytocinergic neurons that send their projections to several brain areas (6). Indeed, to our knowledge, it is unknown whether these oxytocinergic neurons contain NO synthase or not, although the coexistence of the enzyme with vasopressin in magnocellular neurons has been recently reported (9).

In conclusion, the present results suggest that NO via a cGMP-dependent mechanism is involved in the control of penile erection and yawning induced by apomorphine or oxytocin.

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