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Penile Erection and Yawning Induced by Paraventricular NMDA Injection in Male Rats Are Mediated by Oxytocin

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MELIS, M. R., R. STANCAMPIANO AND A. ARGIOLAS. *Penile erection and yawning induced by paraventricular NMDA injection in male rats are mediated by oxytocin.* PHARMACOL BIOCHEM BEHAV **48**(1) 203–207, 1994.—The effect of *N*-methyl-D-aspartic acid (NMDA), (±)-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA), or (±)-trans-1-amino-1,3-cyclo-pentanedicarboxylic acid (ACPD) (5–60 ng in 0.3 μl of saline) microinjected in the paraventricular nucleus of the hypothalamus on penile erection and yawning was studied in male rats. NMDA induced both penile erection and yawning in a dose-dependent manner. AMPA and ACPD also induced penile erection but less potently than NMDA, but were ineffective in causing yawning. NMDA effect on penile erection and yawning was prevented by (+)-MK-801 (0.05–0.1 mg/kg IP, 10 min before NMDA), by the oxytocin antagonist d(CH_2)$_5$Tyr(Me)-Orn^8-vasotocin (50–100 ng ICV 10 min before NMDA), but not by haloperidol (0.1–0.5 mg/kg IP 10 min before NMDA). The results suggest that NMDA induces penile erection and yawning by increasing oxytocinergic transmission by acting in the paraventricular nucleus of the hypothalamus.

Glutamic acid NMDA Oxytocin Penile erection Yawning Paraventricular nucleus Rat

PENILE erection and yawning are two distinct behavioral patterns that often occur concomitantly under certain physiological and experimental conditions [see (9)]. Among substances that induce both penile erection and yawning, dopaminergic agonists oxytocin and adrenocorticotropin (ACTH) are certainly the most widely known. While several lines of experimental evidence suggest that a dopamine–oxytocin link plays a key role in the expression of these behavioral responses [see (16)], ACTH and related peptides seem to act with a mechanism(s) not involving central dopamine or oxytocin (2). Accordingly, both penile erection and yawning are induced by apomorphine, a classical dopaminergic agonist, and by oxytocin injected in the paraventricular nucleus of the hypothalamus (PVN) (14,15), and both responses are prevented either by oxytocin antagonists (16) or by electrolytic lesions of the PVN (1) that deplete oxytocin in the central nervous system (8,10). The above responses seem to be mediated by an influx of Ca^{2+} ions through voltage-dependent Ca^{2+} channels rather than by an increased excitatory amino acid neurotransmission, because they are prevented by the intracerebroventricular (ICV) and PVN injection of ω-conotoxin (3), a potent inhibitor of voltage-dependent Ca^{2+} channels in the nervous tissue (12), but not by several types of excitatory amino acid receptor antagonists (17). However, the above results do not rule out the possibility that excitatory amino acids might control the expression of the above responses by acting before apomorphine and/or oxytocin. Indeed, the PVN receives glutamatergic and/or aspartatergic innervations from several brain areas (29), and recent studies have shown that excitatory amino acids, i.e., kainic acid and *N*-methyl-D-aspartic acid (NMDA) induce yawning and genital grooming when injected in the PVN (25,26). This prompted us to study the effect of agonists of the various excitatory amino acid receptor subtypes [see (20)] when injected in the PVN on penile erection and yawning in male rats to verify whether the above behavioral responses to these excitatory amino acids were mediated by the activation of dopaminergic and/or oxytocinergic transmission in the PVN.

METHOD

Animals

Male Sprague-Dawley rats (200–250 g, Morini, Bologna, Italy) were used in all experiments. Rats were caged in groups

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of 4-6 at 24°C, 60% humidity, with water and standard laboratory food ad lib.

ICV and PVN Injections

Stainless steel guide cannulas (22 gauge) aimed at one lateral ventricle (ICV) or unilaterally at the PVN were stereotaxically implanted under chloral hydrate anaesthesia 5 days before experiments (coordinates: lateral ventricle, 1 mm anterior to bregma, 1.5 mm lateral to midline, and 2 mm ventral to dura; PVN, 0.2 mm anterior to bregma, 0.4 mm lateral to midline, and 2 mm ventral to dura) (23). Animals were allowed 5 days to recover from surgery; each rat was used only once. Oxytocin or d(CH_2)$_5$Tyr(Me)-Orn^8-vasotocin dissolved in saline or saline alone (5 μl in 15 s) were injected ICV via an internal cannula (28 gauge), which 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 microinjections, substances dissolved in saline or saline alone (0.3 μl in 2 min) were injected in the PVN by means of an internal cannula (28 gauge) which extended 5.3 mm below the tip of the guide cannula and connected to a 10 μl Hamilton syringe driven by a Stoelting microinfusion pump. After ICV or PVN injections, 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. Haloperidol was dissolved with a drop of acetic acid, diluted with saline (final pH = 5.5), and injected intraperitoneally (IP) in a volume of 1 ml/200 g body weight. Controls received the same volume of IP vehicle. MK-801 was dissolved in saline and injected IP in a volume of 1 ml/200 g body weight. Controls received the same volume of IP saline.

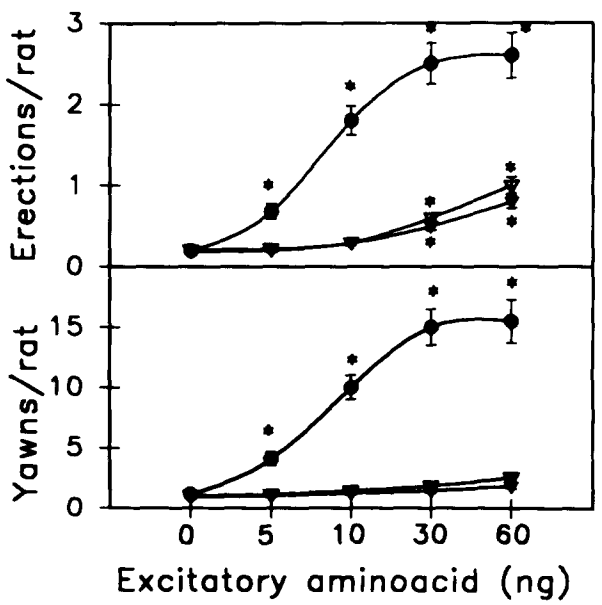

FIG. 1. Effect of NMDA, AMPA, and ACPD microinjected in the PVN in causing penile erection and yawning: dose-response curves. NMDA (●), AMPA (▼), ACPD (▽) was injected in the PVN in a volume of 0.3 μl of saline in 2 min as described in the Method section. Values are mean ± SEM of three experiments (10 rats per group). *$p < 0.01$ with respect to saline-injected rats (μg of excitatory amino acid = 0) (Duncan's multiple range test).

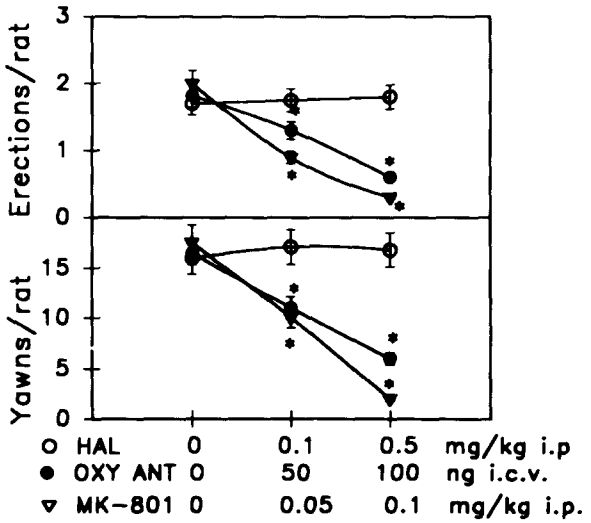

FIG. 2. Effect of MK-801, d(CH_2)$_5$Tyr(Me)-Orn^8-vasotocin and haloperidol on PVN NMDA-induced penile erection and yawning. MK-801 (▼) and haloperidol (○) were given IP and d(CH_2)$_5$Tyr(Me)-Orn^8-vasotocin (●) ICV 10 min before NMDA (30 ng in the PVN) as described in the Method section. Values are mean ± SEM of three experiments (nine rats per group). *$p < 0.01$ with respect to saline-pretreated rats (haloperidol, d(CH_2)$_5$Tyr(Me)-Orn^8-vasotocin or MK-801 = 0) (Duncan's multiple range test).

Behavioral Studies

MK-801 and haloperidol were given IP and the oxytocin antagonist d(CH_2)$_5$Tyr(Me)-Orn^8-vasotocin (4) ICV or in the PVN 10 min before PVN NMDA or SC apomorphine or ICV oxytocin. Shortly after the excitatory amino acid, oxytocin, or apomorphine, the animals were placed individually into Plexiglas cages (30 × 30 × 30 cm) and observed for 60 min, during which the number of penile erections and yawns 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. In those experiments in which PVN microinjections were performed, at the end of experiments animals were killed by decapitation, 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 that were found to have the internal cannula tip positioned correctly in the lateral ventricle or in the PVN were considered for statistical analysis of the results.

Drugs and Peptides

Apomorphine-HCl and *N*-methyl-D-aspartic acid (NMDA), (±)-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA), and (±)-trans-1-amino-1,3-cyclo-pentanedicarboxylic acid (ACPD) were purchased from Sigma (S. Louis, MO),

haloperidol from Janssen, Belgium, (5R,10S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclo-hepten-5,10-iminehydrogen maleate [(+)MK-801] from Research Biochemicals Inc. (Natick, MA), synthetic oxytocin and d(CH$_2$)$_5$Tyr(Me)-Orn^8-vasotocin from Peninsula Laboratories (Palo Alto, CA).

Statistics

Statistical evaluation of the results was performed by one-way ANOVA, followed by Duncan's multiple range test. A $p < 0.05$ was considered significant.

RESULTS

Figure 1 shows the dose-response curve of NMDA, AMPA, or ACPD injected unilaterally in the PVN in causing penile erection and yawning in male rats. NMDA induced both responses in a dose-dependent manner. The minimal effective dose was 5 ng (0.032 nmol), which induced the above responses in 55% of the animals. The maximal effect was found with 30 ng, that induced penile erection and yawning in all rats. Like NMDA, AMPA and ACPD also induced penile erection but to a much lesser extent and only at the highest doses tested. However, unlike NMDA, AMPA and ACPD were unable to induce yawning.

The effect of NMDA (30 ng in the PVN) on penile erection and yawning was prevented in a dose-dependent manner by systemic MK-801 (0.05 and 0.1 mg/kg IP 10 min before NMDA) and by ICV d(CH$_2$)$_5$Tyr(Me)-Orn^8-vasotocin (50 and 100 ng 10 min before NMDA), but not by systemic haloperidol (0.1 and 0.5 mg/kg IP 10 min before NMDA) (Fig. 2). The effect of NMDA was also prevented by MK-801 (50 ng) but not by d(CH$_2$)$_5$Tyr(Me)-Orn^8-vasotocin (100 ng) injected directly in the PVN 10 min before NMDA. In contrast, MK-801 failed to prevent penile erection and yawning induced by oxytocin (30 ng) or by apomorphine (100 ng) injected in the PVN, while d(CH$_2$)$_5$Tyr(Me)-Orn^8-vasotocin prevented these behavioral responses induced by oxytocin but not by apomorphine (Fig. 3).

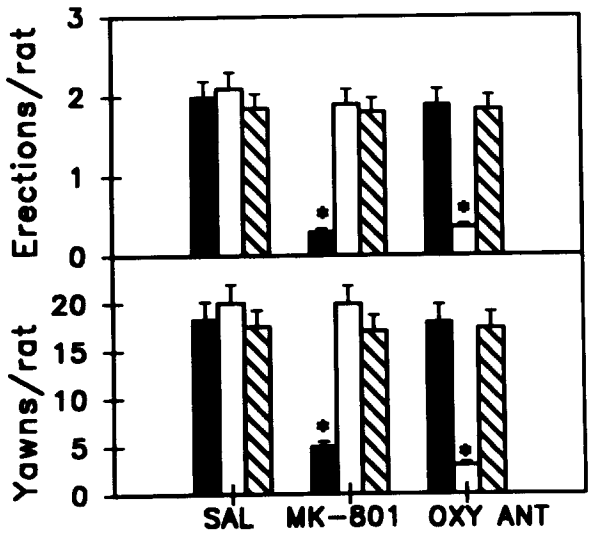

FIG. 3. Effect of MK-801 and d(CH$_2$)$_5$Tyr(Me)-Orn^8-vasotocin microinjected in the PVN on penile erection and yawning induced by NMDA, oxytocin, or apomorphine microinjected in the PVN. Saline (0.3 μl), MK-801 (50 ng/0.3 μl) or d(CH$_2$)$_5$Tyr(Me)-Orn^8-vasotocin (100 ng/0.3 μl) was injected in the PVN 10 min before NMDA (30 ng, filled bars), oxytocin (30 ng, empty bars) or apomorphine (100 ng, diagonal-filled bars) as described in the Method section. Values are mean ± SEM of three experiments (12 rats per group). *$p <$ 0.01 with respect to PVN saline-injected rats (Duncan's multiple range test).

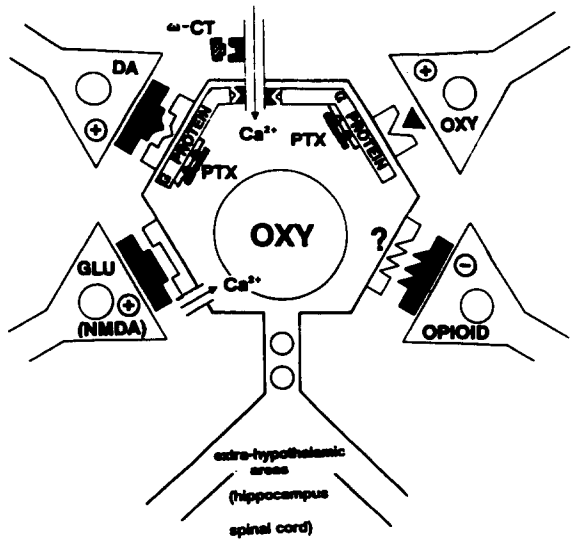

FIG. 4. Oxytocin-induced penile erection and yawning: schematic representation of a hypothetical mechanism of action and interaction with dopaminergic, glutamatergic, and opioid systems in the PVN. According to this model, oxytocin induces penile erection and yawning by activating its own neurons projecting to extrahypothalamic brain areas through the stimulation of specific receptors coupled to ω-conotoxin-sensitive Ca^{2+} channels by a pertussis toxin-sensitive G protein (28). This would cause a influx of Ca^{2+} ions that would act as a second messenger. Likewise, dopaminergic agonists and NMDA would activate oxytocinergic transmission by increasing Ca^{2+} influx through the stimulation of their specific receptors located in oxytocinergic cell bodies. The mechanism by means of which opioids inhibit oxytocinergic transmission is still unknown, but evidence showing that opioid receptors might be located in PVN oxytocinergic cell bodies have been provided [see (19,22)].

DISCUSSION

The present results show that NMDA, a selective agonist of the excitatory amino acid NMDA receptor subtype, but not AMPA or ACPD, agonists of the AMPA and metabotropic receptor subtypes, respectively [see (20)], induces penile erection and yawning when injected in the PVN of male rats. This is in agreement with previous studies showing that NMDA (25) and kainic acid (26) induce yawning and genital grooming when injected in the PVN. Most important, in agreement with the scarce effect of either AMPA or ACPD on penile erection and yawning, NMDA is apparently acting by stimulating specific NMDA receptors, because it is prevented by MK-801, a potent noncompetitive NMDA receptor antagonist (30), given either IP or in the PVN. The activation of these NMDA receptors in the PVN would cause, in turn, the activation of central oxytocinergic transmission, because penile erection and yawning induced by NMDA is prevented by the ICV injection of d(CH$_2$)$_5$Tyr(Me)-Orn^8-vasotocin, a potent oxytocin antagonist (4) (see Fig. 4). However, NMDA effect would not be mediated by the release of oxytocin in the PVN, but in other brain areas, because it is not prevented by the injection of the oxyto-

cin antagonist directly in this hypothalamic nucleus. The possibility that NMDA effect is mediated by the release of dopamine from incertohypothalamic neurons impinging on and activating, in turn, oxytocinergic neurons in the PVN (5,11) is also unlikely. In fact, the blockade of dopaminergic receptors by haloperidol was found to be unable to prevent NMDA-induced penile erection and yawning, thus excluding the existence of a glutamic acid–dopamine–oxytocin link playing a role in the expression of these responses. Nevertheless, the results suggest the existence of a neuronal glutamic acid–oxytocin link involved in the expression of these behavioral responses in addition to the already described dopamine–oxytocin link (16,18). It is likely that the oxytocinergic neurons mediating penile erection and yawning activated by NMDA are the same activated by apomorphine and other dopaminergic agonists, although further studies are necessary to confirm such a possibility.

The molecular mechanism by means of which NMDA activates oxytocinergic transmission is unknown, and only some speculation is possible at present. One possibility is that NMDA induces a Ca^{2+} influx through the well-characterized NMDA receptor-coupled Ca^{2+} channel [see (20)]. In this respect, it is noteworthy that apomorphine and oxytocin effects also seem to be mediated by a Ca^{2+} influx through ω-conotoxin-sensitive Ca^{2+} channels (3). Interestingly, NMDA-induced Ca^{2+} influx seems to be correlated with the activation of nitric oxide (NO)-synthase, the enzyme that produces the novel discovered neurotransmitter/neuromodulator NO [see (27)], and NO-synthase inhibitors prevent apomorphine- and oxytocin-induced penile erection and yawning (13). This raises the possibility that NMDA-, apomorphine-, and oxytocin-induced penile erection and yawning are mediated by an increased production of NO secondary to a common mechanism, e.g., a receptor-induced Ca^{2+} influx (see Fig. 4).

The present study does not support our previous suggestion that excitatory amino acids are not involved in the expression of penile erection and yawning. Our proposal was sustained by the inability of several excitatory amino acid receptor antagonists to prevent apomorphine-, oxytocin-, and ACTH-induced penile erection and yawning when injected ICV or in the PVN (17). The present results, in fact, shows not only that excitatory amino acids are involved but also that they activate directly oxytocinergic transmission to induce these behavioral responses. Nevertheless, the results are in line with those of our previous study, because if one admits that exogenous oxytocin acts on specific oxytocinergic receptors in the PVN to induce penile erection and yawning, excitatory amino acid receptor antagonists would have not prevented oxytocin effect unless mediated by the release of an endogenous excitatory amino acid.

In conclusion, NMDA induces penile erection and yawning when injected in the PVN. This suggests that oxytocinergic neurons mediating these responses are also under the control of excitatory amino acids in addition to other neurotransmitters, i.e., dopamine (16), opioid peptides (19), and oxytocin itself (14), as already described for PVN oxytocinergic magnocellular neurons (5–7,11,21,22,24). In particular, dopamine, glutamic acid, and oxytocin itself seem to have a facilitatory and opioids an inhibitory role in the expression of penile erection and yawning.

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