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Central oxytocinergic and dopaminergic mechanisms regulating penile erection in conscious rats

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Abstract

A series of in vivo studies in a conscious rat model was conducted to investigate the role of oxytocinergic and dopaminergic neurotransmission in the central regulation of penile erection. Oxytocin, when administrated either intracerebroventricularly (i.c.v.) or intrathecally (i.t.) at the spinal levels of L4–L6, produced dose-related erectogenic effects with a maximum at 0.1 μg/rat i.c.v. or 0.03 μg/rat i.t. Oxytocin-evoked penile activity was attenuated by the inhibitory effect of the selective oxytocin antagonist vasotocin analog [Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2 (0.1–1 μg, i.c.v. or i.t.). Penile erection induced by oxytocin was blocked by the dopaminergic receptor antagonist clozapine (1–10 μmol/kg i.p.) in a dose-dependent manner. Conversely, oxytocin antagonist microinjected locally (i.c.v. or i.t.) significantly attenuated the pro-erectile effects of systemic (s.c.) apomorphine, a centrally acting erectogenic agent through dopaminergic receptors. Together, these data indicate a possible concomitant role between dopamine and oxytocin in mediating penile erection at both the spinal and supraspinal sites.

Keywords: Apomorphine; Dopamine; Oxytocin; Penile erection; Intrathecal

1. Introduction

Normal erectile function is an integrated physiological process under control of the central nervous system (CNS) involving supraspinal centers, spinal cord and peripheral nerves (Moreland et al., 2001). Many neurotransmitters and neuromodulators including oxytocin, dopamine, and nitric oxide are involved at a central level in the control of penile erection yet the physiological significance of these agents in the erectile process still remains unclear (Andersson, 2001; Argiolas and Melis, 2004; Melis et al., 1998). Several lines of evidence suggest that central oxytocinergic neurons originating in the paraventricular nucleus (PVN) and projecting to extrahypothalamic brain areas including the hippocampus, ventral medulla and the lumbar–sacral region of the spinal cord may play an important role in the control of penile erection (Argiolas and Melis, 1995, 2004; Giuliano et al., 2001; Lang et al., 1983; Melis and Argiolas, 2003; Melis et al., 1986). Low amounts of oxytocin administered to rats are effective in inducing repeated episodes of penile erections identifying it as one of the most potent substances facilitating penile erection (Argiolas et al., 1986; Melis et al., 1992).

Dopamine neurotransmission also plays an important role in the regulation of penile erection. Apomorphine, a nonselective dopamine agonist, initiates and enhances erectile responses with either systemic or central administration (Hsieh et al., 2004; Rampin, 2001). More recently, it has been demonstrated that selective dopamine D_4 receptor agonists such as PD168077 and ABT-724 produce penile erection in rats (Brioni et al., 2004; Hsieh et al., 2004; Melis et al., 2005). Several lines of evidence indicate that

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dopamine-mediated penile erection involves oxytocinergic recruitment (Argiolas and Melis, 2004; Giuliano and Rampin, 2000; Moreland et al., 2001). Stimulation of D_2-like receptors (D_2, D_3, and D_4) increases oxytocin levels in extra-hypothalamic brain areas leading to activation of the oxytocinergic neurons, potentially evoking penile erection, suggesting a common pathway between oxytocin and dopamine (Heaton, 2000; Melis and Argiolas, 2003).

Although the mechanism by which oxytocinergic neurons act in the CNS to induce penile erection remains unclear, it has been suggested that oxytocin facilitates its own release and excites oxytocin specific neurons (Argiolas and Gessa, 1991; Melis et al., 1986; Melis et al., 1992; Yamashita et al., 1987). It has also been demonstrated that oxytocin released at the lumbosacral spinal cord level by descending projections from the PVN elicit penile erections (Argiolas and Melis, 1995; Giuliano and Rampin, 2000; Rampin, 2001). This pathway represents an efficient and direct pro-erectile link between the supraspinal nuclei and the spinal cord (Giuliano et al., 2001; Melis et al., 1989). Previous studies suggest the existence of a spinal site of action, which may participate in the facilitation of erection (Argiolas and Melis, 1995; Giuliano et al., 2001; Veronneau-Longueville et al., 1999).

While dopaminergic and oxytocinergic neurotransmission at both spinal and supraspinal levels have been previously examined in studying pro-erectile function, there has been little effort to elucidate the co-dependency of these pathways in producing pharmacologically induced penile erection. In the present study, an in vivo conscious rat model was used to investigate the central role of oxytocinergic transmission in the regulation of pro-erectile activity, as well as a possible concomitant role of oxytocin and dopamine in mediating penile erection. Specifically, we examined whether basal i.e. unstimulated D_2-like receptor mediated dopamine neurotransmission was required for pharmacological-induced penile erection produced by either spinal or supraspinal oxytocin receptor activation. Conversely, experiments were carried to examine the role of basal oxytocin receptor activity on penile erections produced by pharmacological activation of D_2-like dopamine receptors. To study the common mechanism for penile erection with these substances, we examined the erectogenic effects of apomorphine (subcutaneous) and oxytocin (i.c.v. or i.t.) and compared the ability of oxytocin and dopamine antagonists ([Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2–vasotocin and clozapine, respectively) to inhibit the erectogenic behavioral responses induced by oxytocin and apomorphine.

2. Methods

2.1. Animals

Male adult Wistar rats, weighing ~300 g (Charles River, Portage Michigan) were used as an animal model to study penile erection in vivo as previously reported (Hsieh et al., 2003). Upon arrival, animals were housed five/cage and given certified food and tap water ad libitum. Acclimation time was one week prior to study start. The housing room was kept at a constant temperature with a 12-h alternating light/dark cycle (light phase 0600–1800 hours) and experiments were carried out between 9:00 a.m. and 3:00 p.m. All experimental protocols and animal handling procedures were approved by the Institutional Animal Care and Use Committee (IACUC, Abbott Laboratories).

2.2. Intracerebroventricular (i.c.v.) surgery

Anesthetized rats (pentobarbital sodium 50 mg/kg i.p.) were placed into a stereotaxic apparatus and a midline incision of approximately 1.5–2.0 cm in length was made longitudinally. A stainless steel guide cannula (22 gauge) was stereotaxically aimed at the left lateral ventricle (stereotaxic coordinates: 1.0 mm posterior to Bregma, 1.6 mm left lateral to midline, and 4.5 mm ventral from the surface of the skull (Paxinos and Watson, 1982). After the skull was cleaned and dried, a small amount of dental acrylic cement was pasted on the surface of the skull so that it covered the skull screws and secured the implantation cannula in place. After the cement was completely dry and hardened, a stainless steel stylet was used to occlude the guide cannula during recovery and between drug injections. The incision was closed using wound clips. The rats were then removed from the stereotaxic apparatus and placed onto a 37 °C warming plate to allow them to recover from anesthesia after surgery. Animals were individually housed and allowed to recover for 7 days before any experimental treatment.

Oxytocin was infused intracerebrally into the lateral ventricle alone (5 μl/min) or in conjunction with its antagonist [Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2–vasotocin (5 μl/min), given i.c.v. 15 min prior to oxytocin. A saline control group was also included in the study. Drugs were administered through a chronic guide cannula. The tip of the injection cannula was left in place for 30 s after dosing to allow for the spread of the injected solutions. After the experiment was completed, cannula placement was confirmed by the infusion of 0.5% fast-green dye in saline solution (5 μl) and subsequent dissection. The same experimentor conducted all i.c.v. surgeries. Success rate was greater than 95%.

2.3. Intrathecal (i.t.) surgery

Procedures were adapted from those described by Yaksh and Rudy (1976). Rats were lightly anesthetized using a halothane (2–5%)/oxygen mixture under proper aseptic conditions and securely mounted onto an intrathecal stereotaxic instrument by placing the animal into blunt ear bars, to secure the animal's head firmly in place. A

small incision was made vertically from the dorsal surface of the occipital bone to the base of the skull (2 cm). Tissue was then displaced using a blunt probe so that the atlanto-occipital membrane at the base of the skull was clearly seen. A shallow (1 mm) slit was made in the atlanto-occipital membrane and a single intrathecal catheter (PE5—8.5 cm/PE10—4 cm; external portion, University of San Diego, CA) was inserted caudally into the rat spinal subarachnoid space with the caudal tip of the PE5 catheter at the L4–L6 spinal level (LoPachin et al., 1981). The PE5 tubing was slowly and gently inserted from the incision point to the lumbar enlargement (L4–L6). Once the notch rested on the atlanto-occipital membrane (indicating the tip is in the lumbar enlargement), an 18-gauge needle tip was slid through the posterior (to the initial incision) skin surface. With the needle remaining in the skin, the external portion of the catheter was threaded into the needle. Both the needle and the external catheter were pulled caudally through the skin to keep the catheter in place and out of reach of the animal. A 4.0 nylon suture was used to close the muscle incision and secure the catheter. The incision was closed with surgical wound clips. The catheter was filled with sterile water and the ends of the catheter were heat-sealed.

Following the recovery from surgery, animals were individually housed. If motor impairment was noticed, the animals were immediately euthanized. Animals with catheters were allowed at least 1 week of recovery from surgery prior to behavioral testing. For compound intrathecal injection, a Hamilton syringe (50 μl) was connected to the external portion (4 cm) of the catheter and 5–10 μl/rat of either oxytocin or [Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2–vasotocin (given 15 min prior to oxytocin) was slowly injected into the catheter. A saline control group was also included in the studies. Drug injection was followed by 10 μl saline flush to assure the drug was delivered to the L4–L6 region of the spinal cord. The tip of the catheter was then cauterized. Using the fast-green dyed saline solution demonstrated that, under this condition, the diffusion of the injection solution was restricted to the spinal areas of the injection site.

2.4. Behavioral test

On the day of testing, animals were allowed to adapt to a testing room diffusely illuminated by red light for 1 h prior to the start of the experiment. Rats were placed individually into a transparent Plexiglas cage (20 × 30 × 30 cm) immediately after drug injection. A mirror was placed behind and under the observation cages to facilitate observation of the animals. Each rat was used only once. A rat penile erection was considered to occur when the following behaviors were presented: repeated pelvic thrusts immediately followed by an upright position and an emerging, engorged penis, followed by genital grooming. (Heaton et al., 1991; Melis et al., 2004). Not all behaviors were necessary to be considered a penile erectile event; only an emerging engorged penis was needed.

2.5. Compounds

Oxytocin and the selective oxytocin antagonist vasotocin analog [Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2 (American Peptide Company Inc., Sunnyvale, CA) were dissolved in 0.9% NaCl and kept on ice for the duration of the studies. Apomorphine–HCL, 1 ml/kg, s.c. (Aldrich Chemical Company, Milwaukee, WI) was dissolved in 1 mg of ascorbic acid/1 ml saline. Clozapine (Sigma, St. Louis, MO) was dissolved with 0.05% acetic acid and diluted in 1 mg ascorbic acid/1 ml saline. All compounds were prepared fresh daily.

2.6. Statistical analysis

The penile erection episodes were recorded by direct observation for a period of 60 min following the compound dosing and erection incidence (%) was defined as the percentage of animals exhibiting one or more erections during the observation period. Data were expressed as incidence (%) ± S.E. calculated by the use of a Wald equation (Dowdy and Wearden, 1983). Statistical evaluation of the results was performed by a chi-square test. A $p < 0.05$ was considered significant. The number of penile erections was also counted and the data, expressed as mean ± S.E.M. of erections over the observation period, were analyzed by the Mann–Whitney nonparametric test. Significance was set at $p < 0.05$.

3. Results

Oxytocin administered i.c.v. facilitated penile erection in a dose-dependent manner with maximum effects at 0.01–0.1 μg/rat (87%, $p < 0.01$ erectile response compared to vehicle control) (Fig. 1A). A dose of 0.1 μg was chosen for oxytocin i.c.v. administration in all subsequent studies. This dose also corresponded to the greatest number of penile episodes per rat, with an overall average of 4 erections ± 0.8 (median = 5, $p < 0.01$ vs. vehicle control). When the oxytocin antagonist [Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2–vasotocin was given i.c.v. 15 min prior to oxytocin i.c.v. (0.1 μg), it significantly and dose-dependently reduced the erectogenic effect of oxytocin. The minimal effective dose of 0.1 μg [Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2–vasotocin reduced the erectile response by 30% ($p < 0.05$) when compared to the oxytocin-injected group, while the erectile response was reduced by 70% ($p < 0.001$), vs. the oxytocin group, with the highest dose given at 1 μg (Fig. 1B).

To determine the pro-erectile effects of oxytocin when given i.t., a dose–response curve was generated and oxytocin was again effective, significantly increasing penile

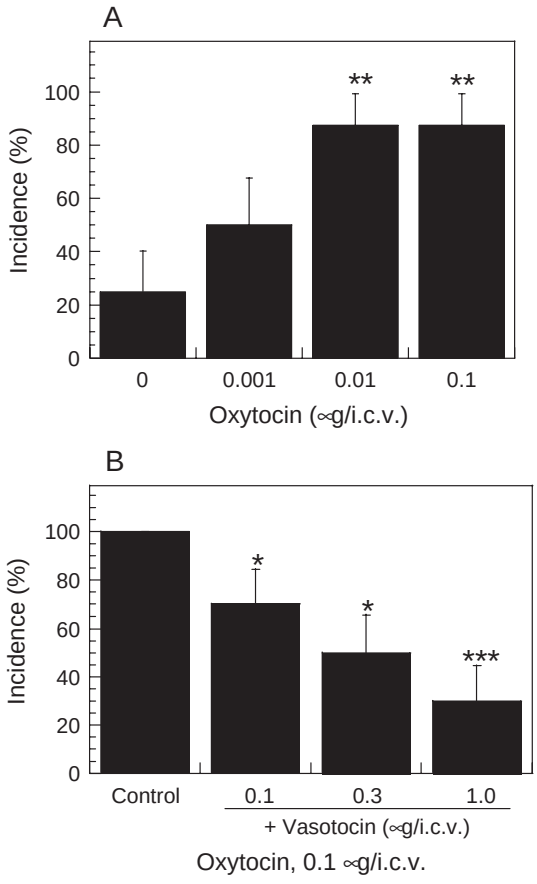

Fig. 1. Pro-erectile effects of i.c.v. microinjections of oxytocin ($n=8-10$) and blockade effects with i.c.v. microinjections of the oxytocin antagonist [Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2–vasotocin in conscious rats. Oxytocin has significant effects in the rat penile erection (RPE) assay in a dose dependent manner (A). [Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2–vasotocin is effective in blocking the erectogenic effects of oxytocin (0.1 μg) when administered 15 min prior to oxytocin (B). Drugs were injected at 5 μl/min and the infusion cannulae were removed 30 s after dosing. Data are expressed as erection incidence (%)±S.E. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ vs. vehicle (A) or oxytocin (B).

erections with significance seen at doses ranging from 0.003–0.3 μg (Fig. 2A). A dose of 0.03 μg was the chosen dose for oxytocin i.t. administration in all of the remaining studies. This dose also corresponded to the greatest number of penile episodes per rat, with an overall average of 2±0.55 erections (median=2, $p<0.01$ vs. vehicle control). [Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2–vasotocin was given i.t., 15 min prior to i.t. injection of oxytocin (0.03 μg/rat), which effectively blocked pro-erectile oxytocinergic effects. The minimal effective dose of 0.1 μg decreased erectile responses by 43% ($p<0.05$) when compared to the oxytocin-injected group, while both 0.3 and 1.0 μg doses significantly decreased erections by 86% ($p<0.001$) vs. the oxytocin group (Fig. 2B).

To investigate the role of dopamine in the activation of oxytocinergic neurons for erectogenic response, the D_2-like antagonist, clozapine, was given i.p., 1-h prior to oxytocin administered i.c.v. (0.1 μg) or i.t. (0.03 μg). Clozapine attenuated the erectile response at 10 μmol/kg, i.p. showing 85% decrease ($p<0.001$) when compared to the 0.1 μg, i.c.v. oxytocin-injected group (Fig. 3A) and 79% ($p<0.05$) decrease was also seen when compared to 0.03 μg, i.t. oxytocin-injected group (Fig. 3B).

To further demonstrate the concomitant role between dopamine and oxytocin in penile erection, subcutaneous apomorphine (0.1 μmol/kg), which has been previously shown to induce optimal erectogenic activity (Hsieh et al., 2004), was administered 15 min after [Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2–vasotocin i.c.v or i.t. Significant blockade was seen with the [Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2 –vasotocin pre-treatment of 1.0 μg/i.c.v., showing a 78% erectile decrease ($p<0.001$) when compared to the apomorphine-injected group, (Fig. 4A), and when [Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2–vasotocin was administered at 0.3 μg/i.t. a 66%

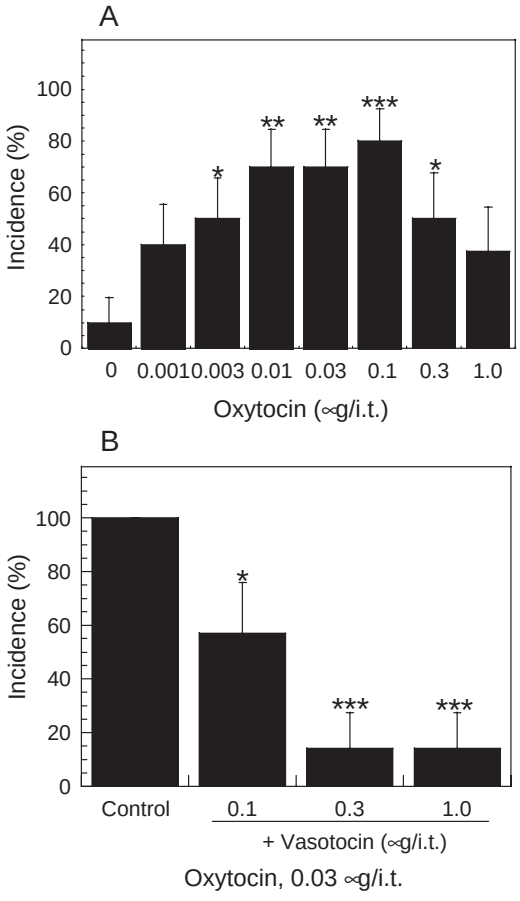

Fig. 2. Pro-erectile effects of i.t. microinjections of oxytocin ($n=7-10$) and blockade effects with i.c.v. microinjections of the oxytocin antagonist [Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2–vasotocin in conscious rats. Oxytocin has significant effects in the rat penile erection (RPE) assay when administered intrathecally (A). [Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2–vasotocin significantly blocks oxytocin's erectogenic effects when given intrathecally 15 min prior to oxytocin (0.03 μg) (B). Drugs were administered at 5–10 μl followed by a saline flush of 10 μl. Data are expressed as erection incidence (%)±S.E. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ vs. vehicle (A) or oxytocin (B).

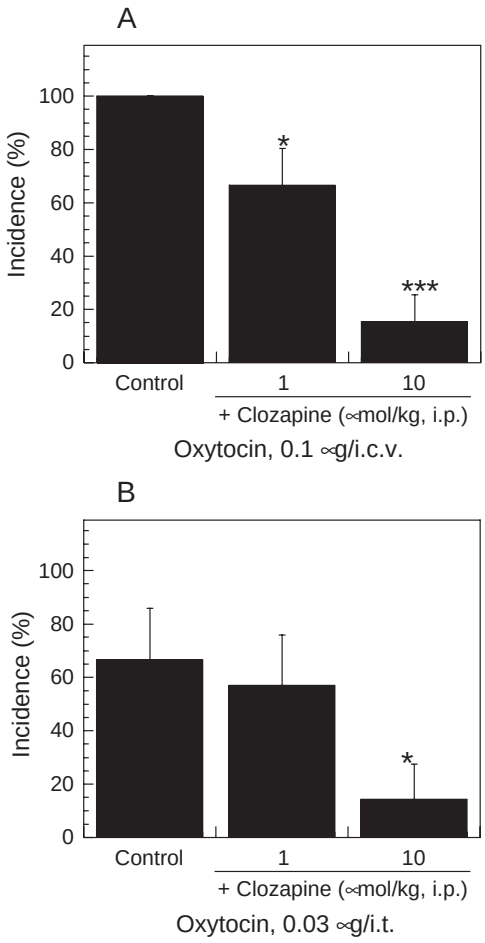

Fig. 3. Systemic clozapine inhibits effects on oxytocin-induced penile erection in conscious rats. Clozapine i.p. significantly blocks oxytocin administered i.c.v. ($n=10-12$, 0.1 μg) (A) or i.t. ($n=6-7$, 0.03 μg) (B) in a dose-dependent manner. Clozapine was given 1 h prior to the oxytocin injection. Data are expressed as erection incidence (%)±S.E. *$p<0.05$ and ***$p<0.001$ vs. vehicle.

erectile decrease ($p<0.01$) was seen when compared to the apomorphine-injected group (Fig. 4B).

4. Discussion

In addition to demonstrating spinal and supraspinal sites of action for oxytocin-mediated penile erection activity, the present results suggest co-involvement of D_2-like dopamine and oxytocin receptors in eliciting penile erection. Specifically, we demonstrated that the penile erection activity observed with exogenous administration of either oxytocin or apomorphine was dependent on basal activity of dopaminergic and oxytocinergic pathways, respectively, as supported by antagonism studies with either site-specific oxytocin receptor antagonism produced by [Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2–vasotocin or central D_2-like dopamine receptor antagonism produced by systemic clozapine. Using this type of "reciprocal" approach, the results of our studies suggest that pharmacological-induced penile erection produced by either oxytocin or D_2-like dopamine receptor activation is dependent on the basal activity of the unstimulated pathway.

While clozapine blocks activation of all three human dopamine D_2-like receptors (Moreland et al., 2004a), ce:inf> receptor preferring/ce:inf> receptor preferring antagonist (Seeman and Van Tol, 1994). Moreover, when the rat dopamine D_{2L} and D_4 receptors are co-expressed with $G\alpha_{qo5}$ in HEK-293 cells and examined in calcium flux assays, clozapine blocks the effects of dopamine at dopamine D_4, but not D_{2L}, receptors (Moreland et al., submitted for publication). These observations are consistent with clozapine blockade of apomorphine-induced penile erection and selective dopamine D_4 agonists inducing penile erection, suggesting a role for the dopamine D_4 receptor in penile erection (Brioni et al., 2004; Hsieh et al., 2004).

The role of central oxytocin on penile erection was first reported in 1985 with studies showing that oxytocin given

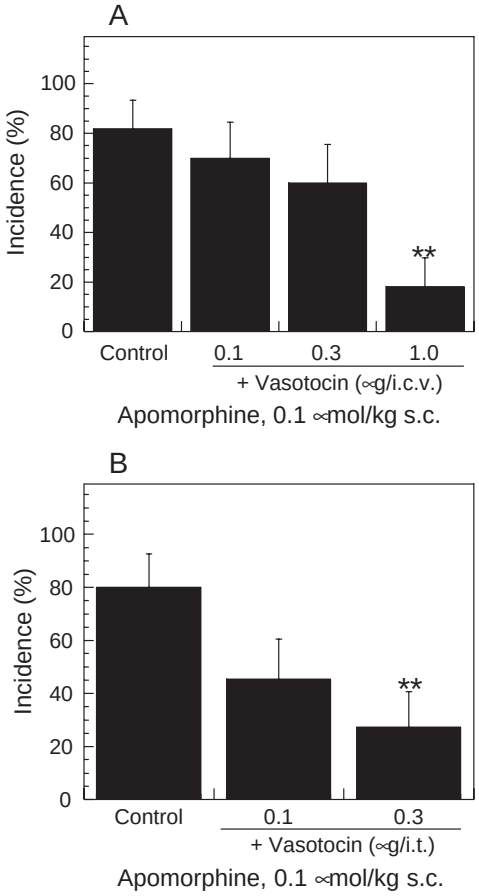

Fig. 4. Central administration of [Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2–vasotocin on apomorphine-induced penile erection. In conscious rats, vasotocin administered i.c.v. ($n=10-11$) (A) or i.t. ($n=7-8$) (B), is effective in blocking the erectogenic effects of apomorphine (0.1 μmol/kg, s.c.). [Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2–vasotocin was administered 10–15 min prior to injection of apomorphine (0.1 μmol/kg). Data are expressed as erection incidence (%)±S.E. **$p<0.01$ vs. apomorphine.

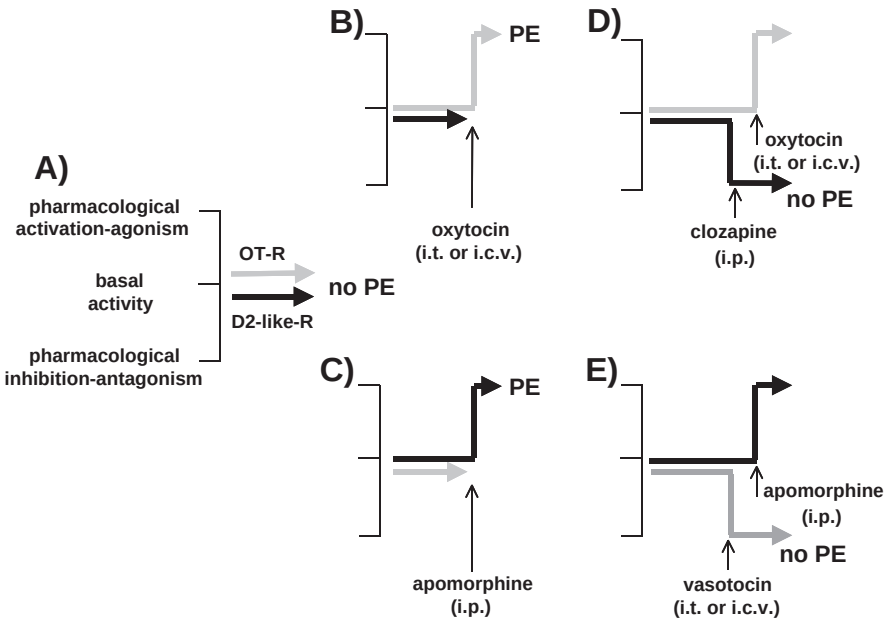

Fig. 5. Interaction between oxytocin and dopamine: parallel dependent pathways eliciting penile erection. (A) Normal basal activity of oxytocin receptor (OT-R) and D2-like receptor (D2-like-R) without pharmacological activation and/or inhibition does not produce penile erection. (B) Pharmacological activation of OT-R with exogenous oxytocin given i.t. or i.c.v. elicits penile response. (C) Pharmacological activation of D_2-like-R with exogenous apomorphine elicits penile response. (D) Pharmacological inhibition with the dopamine antagonist clozapine (i.p. 1 h pre-treatment) with the addition of agonist oxytocin does not produce penile erection. (E) Pharmacological inhibition with the oxytocin antagonist [Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2 –vasotocin (i.t. or i.c.v. 15 min pre-treatment) with the addition of agonist apomorphine does not produce penile erection.

i.c.v. induces penile erection in conscious rats (Argiolas et al., 1985). Comparable effects have also been reported in anesthetized rats (Giuliano et al., 2001). In the present studies, i.c.v. administration of oxytocin in rats produced a dose-dependent induction of penile erection, which was blocked by i.c.v. pre-treatment with the selective oxytocin receptor antagonist [Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2–vasotocin. Previous studies have supported the physiological role of oxytocinergic neurons located in the hypothalamic PVN in mediating control of the erectile response through stimulated release of oxytocin leading to penile erection (Melis and Argiolas, 2003; Giuliano et al., 2001; Argiolas et al., 1988). However, oxytocin-deficient transgenic mice have normal genitalia, normal testicular histology, and are healthy and able to reproduce (Nishimori et al., 1996). While these results suggest that oxytocin is not essential for male reproductive behavior, the role of oxytocin in male sexual function remains to be defined.

Behavioral and electrophysiological studies have shown the PVN to be one of the most sensitive brain areas in the regulation of pro-erectile activity (Giuliano and Rampin, 2000). Oxytocinergic neurons from the PVN project to the extrahypothalamic brain areas including the hippocampus, medulla oblongata, frontal cortex, brainstem and spinal cord (Lang et al., 1983; Sofroniew, 1983). Therefore, it seems plausible that oxytocinergic neurons in the PVN project to higher brain areas, as well as descending pathways to the spinal cord, in mediating penile erection.

Furthermore, i.t. administration of oxytocin produced dose-dependent penile erection activity in conscious rats, which was blocked by i.t. [Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2–vasotocin pre-treatment (Fig. 2). These results in awake rats expand upon previous studies demonstrating in anesthetized rats that oxytocin delivered directly to the lumbar–sacral region of the spinal cord produces pro-erectile responses (Giuliano et al., 2001). Oxytocin-binding sites are present in the sacral parasympathetic nucleus and dorsal gray commissure (DGC) of the L6-S1 spinal cord (Veronneau-Longueville et al., 1999). The DGC is a good candidate for the regulation of penile erection through integration of information from the peripheral and supraspinal origins, redistribution of information to autonomic and somatic neurons and local regulation by a variety of neuromediators including oxytocin and dopamine (Veronneau-Longueville et al., 1999). Activation of spinal or supraspinal neurons by oxytocin or dopamine facilitates penile erection (Melis and Argiolas, 2003; Rampin, 2001; Argiolas, 1994; Giuliano and Rampin, 2000; Veronneau-Longueville et al., 1999). Therefore, the similar efficacy observed with either i.t. or i.c.v. oxytocin, as reported here, may suggest that oxytocin plays equal importance in both the brain and spinal cord in regulating penile erection.

The current data also expand upon the mechanism of central oxytocin in eliciting penile erections by supporting an interaction between dopaminergic and oxytocinergic neurotransmission. Specifically, penile erections produced by both i.t. and i.c.v. oxytocin administration were blocked by systemic pre-treatment with clozapine, indicating a potential role of D_2-like, or more specifically rat dopamine

D_4-preferring, and oxytocin receptor activation in eliciting penile responses. Using dopamine D_4 receptor selective ligand A-369508, no specific binding could be demonstrated in the rat spinal cord (Moreland et al., 2004b). Using quantitative autoradiography, it has been demonstrated that both dopamine D_2 and D_3 receptors are located in the spinal cord (Levant and McCarson, 2001). Therefore, blockade of i.t. oxytocin-induced penile erection by systemic administered clozapine may involve direct antagonism of D_4 receptors in the spinal cord. With regard to supraspinal regulation, clozapine blockade of i.c.v. oxytocin-induced penile erection may involve D_2-like, or specifically dopamine D_4, receptors located in the PVN, as well as other hypothalamic and/or extrahypothalamic brain regions that mediate penile erection activity produced by oxytocin. Although the level of D_2-like receptors in the spinal cord may be lower than in the brain, studies have indicated that activation of spinal cord D_2-like receptors have important functional consequences in autonomic regulation of oxytocin-mediated sexual behavior (Van Dijken et al., 1996).

Previous in vitro and in vivo studies have shown that DA has an excitatory role in oxytocin release (Melis et al., 1989). Although this may suggest that stimulation of dopamine D_2-like receptors leads to activation of oxytocinergic neurons in mediating penile erection, in our findings, the ability of clozapine to block oxytocin-induced penile erection would suggest independent but equally required stimulation of both the oxytocin and dopamine pathways.

While the current data show that clozapine blocks the excitatory effects of oxytocin on pro-erectogenic responses, results of our studies also indicate that the converse is true; that is, oxytocin receptor blockade diminishes the effects of dopamine agonist-induced erectile behavior. Specifically, penile erection activity produced by peripheral administration of the nonselective DA agonist apomorphine was blocked by either i.t. or i.c.v. pre-treatment with [Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2–vasotocin, further supporting the potential facilitative interaction between oxytocin and dopamine at both spinal and supraspinal sites.

Taken together, the results of the present studies suggest the parallel participation of oxytocinergic and dopaminergic pathways in the regulation of penile erection at both the spinal and supraspinal sites. As illustrated in Fig. 5, pharmacological activation of either oxytocin (Fig. 5B) or D_2-like dopamine (Fig. 5C) receptors, specifically dopamine D_4, results in penile erection activity. In contrast, pharmacological antagonism of D_2-like receptors prevents penile erection induction produced by oxytocin receptor activation (Fig. 5D), while conversely oxytocin receptor blockade prevents penile erection induction produced by D_2-like dopamine receptor activation (Fig. 5E).

These findings argue against serial pathways where one neurochemical system subsequently stimulates the other in order to produce penile erection induction. Indeed, if oxytocinergic transmission was upstream from a dopamine pathway, oxytocin receptor antagonism should not block penile erection induction produced by downstream D_2-like activation. Alternatively, if dopaminergic transmission was upstream from an oxytocin pathway, D_2-like dopamine receptor antagonism should not block penile erection induction produced by downstream oxytocinergic activation. Results of these studies, however, demonstrated that [Pmp-Tyr(Me)-Ile-Thr-Asn-Cys]-Pro-Orn-Tyr–NH_2–vasotocin blocked apomorphine-induced penile erection, while clozapine blocked oxytocin-induced penile erection, suggesting parallel-dependency between dopamine D_2-like and oxytocin receptors for producing pro-erectile activity.

In conclusion, the current studies confirm the central role of oxytocin in eliciting erectile function at both the supraspinal and spinal sites of action, suggesting that oxytocin has similar pharmacological efficacy in the brain and spinal cord. These results also indicate that oxytocinergic-mediated penile erection at both sites requires basal D_2-like DA receptor activity for oxytocin pro-erectile activity. Conversely, the pro-erectile activity associated with dopamine D_2-like receptor activation may be dependent on spinal and supraspinal oxytocin receptors. Taken together, these results provide pharmacological and anatomical evidence implicating the requirement of both oxytocinergic and dopaminergic neurotransmission in the central regulation of penile erection.

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