

# Identification, localization and functional *in vitro* and *in vivo* activity of oxytocin receptor in the rat penis

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## Abstract

We recently found that the oxytocin receptor (OTR) is expressed in the human and rabbit corpus cavernosum and mediates contractility *in vitro*. The present study extended our investigations to the rat, and explored whether OTR regulates penile detumescence *in vivo*. Real-time RT-PCR quantitatively characterized the distribution of OTR mRNA in the male genital tract. Specific transcripts for OTR were expressed in all the tissues investigated. Penile expression of OTR was comparable to that observed in testis and prostate. Western blot analysis detected a single band of the expected molecular mass for OTR in all tissues examined, including rat penis. Expression of OTR protein in rat penile extracts was further confirmed by binding studies, using the OTR selective radiolabeled ligand <sup>125</sup>I-OTA ( $K_d = 17 \pm 6.5$  pM,  $B_{max} = 15.7 \pm 5$  fmoles/mg protein). OTR was immunolocalized to the endothelial and smooth muscle compartments of cavernous spaces and

blood vessels. In rat corpus cavernosum strips, oxytocin (OT) and an OTR selective agonist ([Thr<sup>4</sup>,Gly<sup>7</sup>]OT) induced identical increases in tension, while different vasopressin agonists were less active. *In vivo*, OT intracavernous injection (ICI) dose-dependently inhibited intracavernous pressure (ICP) increase elicited by either electrical stimulation of the cavernous nerve or ICI of papaverine with similar  $IC_{50}$ s ( $117.7 \pm 37$  mU). The OTR antagonist, atosiban, counteracted the contractile effect of OT both *in vitro* and *in vivo*. Atosiban alone significantly increased ICP at lower stimulation frequencies (2 Hz =  $P < 0.001$  and 4 Hz =  $P < 0.05$  vs control), but not at the maximal frequency (16 Hz). Our data showed that OTR is present in the rat penis and mediates contractility both *in vitro* and *in vivo*, therefore suggesting a role for OT in maintaining penile detumescence.

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## Introduction

The penis resides in the flaccid, non-erect, state for the majority of the time, due to an active contraction of smooth muscle cells (SMC) in penile vessels and lacunar spaces. Penile SMC contraction is favored by a rapid breakdown of the relaxing, nitric oxide-related, cGMP, through an increased penile expression of phosphodiesterase type 5 (Morelli *et al.* 2004), and by the activity of a complex array of contractile neurotransmitters and neuro-modulators, which has only partly been characterized. It is generally assumed that the release of noradrenaline from sympathetic adrenergic terminals plays a primary role in determining vasoconstriction in penile vascular beds (Maggi *et al.* 2000, Andersson 2003). However, modulatory peptides such as endothelin-1 might also participate in this process, in both physiological (Granchi *et al.* 2002) and pathological (Filippi *et al.* 2003a) conditions. Interestingly, we recently described the presence of the oxytocin

receptor (OTR) gene and protein in rabbit and human cavernous tissue in a similar concentration to that found in other portions of the male genital tract (Vignozzi *et al.* 2004), classically considered the main male target of oxytocin (OT), such as the epididymis (Maggi *et al.* 1987, Einspanier & Ivell 1997, Ivell *et al.* 1997, Frayne & Nicholson 1998, Filippi *et al.* 2002a,b). In the epididymis, OTR mediates an increase in both *in vitro* (Hib 1974, Filippi *et al.* 2002a,b, 2003b) and *in vivo* (Cross 1955, Hib 1977, Nicholson *et al.* 1999, Assinder *et al.* 2002, Studdard *et al.* 2002) contractility and sperm output. Also in the penis OTR mediates *in vitro* contractility and therefore we hypothesized that OT participates in maintaining penile SMC contraction and flaccidity (Vignozzi *et al.* 2004).

OT was originally characterized as a hormone with a permissive role in female reproduction: facilitating uterine contraction and milk ejection (Cross 1955, Soloff & Swartz 1974, Roberts *et al.* 1976, Markle *et al.* 1978, Soloff *et al.* 1979, Filippi *et al.* 2003b). In males, OT was found

to be one of the most potent agents to induce penile erection by electrical or pharmacological stimulation of paraventricular nuclei in rats, rabbits and monkeys (see Argiolas 1992 and Gimpl & Fahrenholz 2001 for reviews). Nevertheless, an additional and peripheral role for OT has been proposed. In fact, in all the studies performed so far (Ogawa *et al.* 1980, Carmichael *et al.* 1987, Murphy *et al.* 1987, Uckert *et al.* 2003), a clear-cut surge of OT plasma levels has been described during male sexual activity, peaking during orgasm and detumescence, with still unknown physiological significance. Since OT cannot easily cross the blood–brain barrier, a central effect of this peptide released by the posterior pituitary during orgasm is rather unlikely. Conversely, a peripheral action of OT should be considered, in particular at the level of the male genital tract.

The primary goal of the present study was to extend our previous observations obtained in other species (Vignozzi *et al.* 2004) to the rat and to verify whether or not OT has any effect in an *in vivo* model of penile erection. Hence, we investigated the pharmacological stimulation with OT or inhibition with atosiban (an OTR antagonist) of OTR in rat corpus cavernosum (CC) on intracavernous pressure (ICP) elicited by electrostimulation (ES) of the cavernous nerve and intracavernous injection (ICI) of papaverine. Essentially, we found that local administration of OT decreased and atosiban increased rat penile erectile response.

## Materials and Methods

### Chemicals and tissues

Phenylephrine HCl, papaverine HCl, oxytocin, [Thr<sup>4</sup>,Gly<sup>7</sup>]oxytocin, [deamino-Cys<sup>1</sup>,D-Arg<sup>8</sup>]vasopressin (DDAVP), reagents for immunocytochemistry, for SDS-PAGE and peroxidase-conjugated anti-mouse secondary antibody were purchased from Sigma (St Louis, MO, USA). (d(CH<sub>2</sub>)<sub>5</sub><sup>1</sup>,Tyr(Me)<sup>2</sup>,Orn<sup>8</sup>]vasotocin (OTA) and (Phe<sup>2</sup>,Orn<sup>8</sup>)vasotocin were purchased from Bachem AG (Bubendorf, Switzerland). d(CH<sub>2</sub>)<sub>5</sub>[Tyr(Me)<sup>2</sup>,Thr<sup>4</sup>,Orn<sup>8</sup>,<sup>125</sup>I-Tyr<sup>9</sup>-NH<sub>2</sub>]-vasotocin (<sup>125</sup>I-OTA) was purchased from NEN Life Science (Boston, MA, USA). Atosiban was from Ferring AB (Limhamn, Sweden). Male rat genital tissues including CC, brain, liver, cornea and uterus were obtained from adult Sprague–Dawley (SD) rats (Harlan, S. Pietro al Natisone, Italy). The Local Ethical Committee for Investigations in Animals of the University of Florence approved the study. Human CC samples were from patients undergoing penile prosthesis implantation (*n*=4) with the approval of the Hospital Committee for Investigation in Humans and with informed consent.

### Isolation of RNA and cDNA synthesis

Total RNA was extracted from frozen tissues using TRIzol (Invitrogen, San Diego, CA, USA) according to

the manufacturer's instructions. RNA concentration and quality were measured by spectrophotometric analysis at 260 and 280 nm. RNA integrity was assessed by electrophoresis in agarose gel. For each sample 400 ng total RNA were reverse-transcribed to cDNA in a final volume of 80 µl using a TaqMan Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA) under the following conditions: 10 min at 25 °C, 30 min at 48 °C, 5 min at 95 °C.

### Real-time quantitative RT-PCR

The mRNA quantitative analysis was performed according to the fluorescent TaqMan methodology as already published (Vignozzi *et al.* 2004). PCR primers and probe for OTR mRNA quantitation were purchased as an Assay-On-Demand (AoD) gene expression product from Applied Biosystems. The beta-2 microglobulin gene was chosen as the reference gene and the corresponding AoD product was provided by Applied Biosystems. The PCR mixture (25 µl final volume) consisted of 1 × final concentration of AoD mix, 1 × final concentration of Universal PCR Master Mix (Applied Biosystems), and 25 ng cDNA. Amplification and detection were performed with the ABI Prism 7700 Sequence Detection System with the following thermal cycler conditions: 2 min at 50 °C, 10 min at 95 °C, and 40 cycles at 95 °C for 30 s and 60 °C for 1 min. Each measurement was carried out in duplicate. The analysis of the results was based on the comparative Ct method according to the manufacturer's instructions (Applied Biosystems), where Ct represents the cycle number at which the fluorescent signal, associated with an exponential increase in PCR products, crossed a given threshold. The maximum change in Ct values of the sample (Ct<sub>sample</sub>) was determined by subtracting the average of duplicate Ct values of the reference gene from the average of duplicate Ct values of the target gene.

### Immunohistochemistry

Immunohistochemical studies were carried out as previously described (Maggi *et al.* 1991, Vignozzi *et al.* 2004) in both rat CC and mammary gland. Briefly, rat penile and breast sections (fixed in Bouin's solution and embedded in paraffin) were subsequently incubated, first for 1 h in 2% FCS in PBS to block non-specific antibody binding, overnight at 4 °C with the IgM mouse CHINA/1F3 (diluted 1:200), then with the corresponding specific immunoglobulin peroxidase conjugates for 30 min (dilution 1:1000). Demonstration of peroxidase activity and controls for specificity of the antisera were performed as previously described (Maggi *et al.* 1991, Vignozzi *et al.* 2004). The specificity of the anti-OTR antibodies in this study was controlled by (1) omission of the primary antibodies and (2) pre-absorption of the primary antibodies with myometrial cell microsomes, expressing a high

density (15 pmoles/mg protein) of OTR (Maggi *et al.* 1994a). CHINA/1F3 antibodies (working dilution) were incubated with 1 mg/ml myometrial membranes overnight at 4 °C. After an additional 60 min incubation with 4% polyethylene glycol, the unbound antibodies were separated by rapid centrifugation and used for immunohistochemistry. The slides were photographed using a Nikon Microphot-FX microscope (Nikon, Kogaku, Tokyo, Japan).

#### SDS-PAGE and Western blot analysis

Protein from male rat reproductive tissues, liver, uterus and human CC were prepared as previously described (Vignozzi *et al.* 2004). Briefly, frozen samples were homogenized in lysis buffer. Aliquots containing 30 µg proteins were diluted in reducing 2 × SB (Laemmli's sample buffer) and loaded onto 10% SDS-PAGE. After separation, proteins were transferred to nitrocellulose membranes. Membranes were blocked for 2 h at room temperature in 10% BM blocking buffer (BM, Roche Diagnostics, Milan, Italy)-TTBS (0.1% Tween-20, 20 mM Tris, 150 mM NaCl), washed in TTBS and incubated overnight with anti-OTR CHINA/1F3 antibody (1:1000 in BM blocking buffer-TTBS) followed by peroxidase-conjugated secondary IgG (1:3000). Finally, reacted proteins were revealed by the BM enhanced-chemiluminescence system (Roche Diagnostics).

#### Membrane preparation and binding studies

Membranes from rat CC were prepared as previously described (Maggi *et al.* 1987, 1994b, Peri *et al.* 1997, 1998, Vignozzi *et al.* 2004). Briefly, aliquots of membranes (0.075 mg/ml) were incubated (in a final volume, 0.25 ml) with <sup>125</sup>I-OTA (15–50 pM), in a buffer containing 50 mM Tris-maleate, pH 7.6 with 10 mM MgSO<sub>4</sub>, 1 mM benzamidine, 0.01% bacitracin, and 0.002% soy-bean trypsin inhibitor in the presence of 0.1% BSA at 22 °C for 60 min with or without increasing concentrations of various unlabeled compounds: OTA (the corresponding unlabeled peptide), OT, (Phe<sup>2</sup>, Orn<sup>8</sup>)VT (a selective V<sub>1</sub> vasopressin agonist) and DDAVP (the selective V<sub>2</sub> vasopressin agonist). All measurements were performed in triplicate. Then, membrane suspensions were filtered through Whatman GF/B filters by using the Brandel M-48R. Radioactivity was counted in a γ-counter. Results were simultaneously fitted using the LIGAND program (Munson & Rodbard 1980).

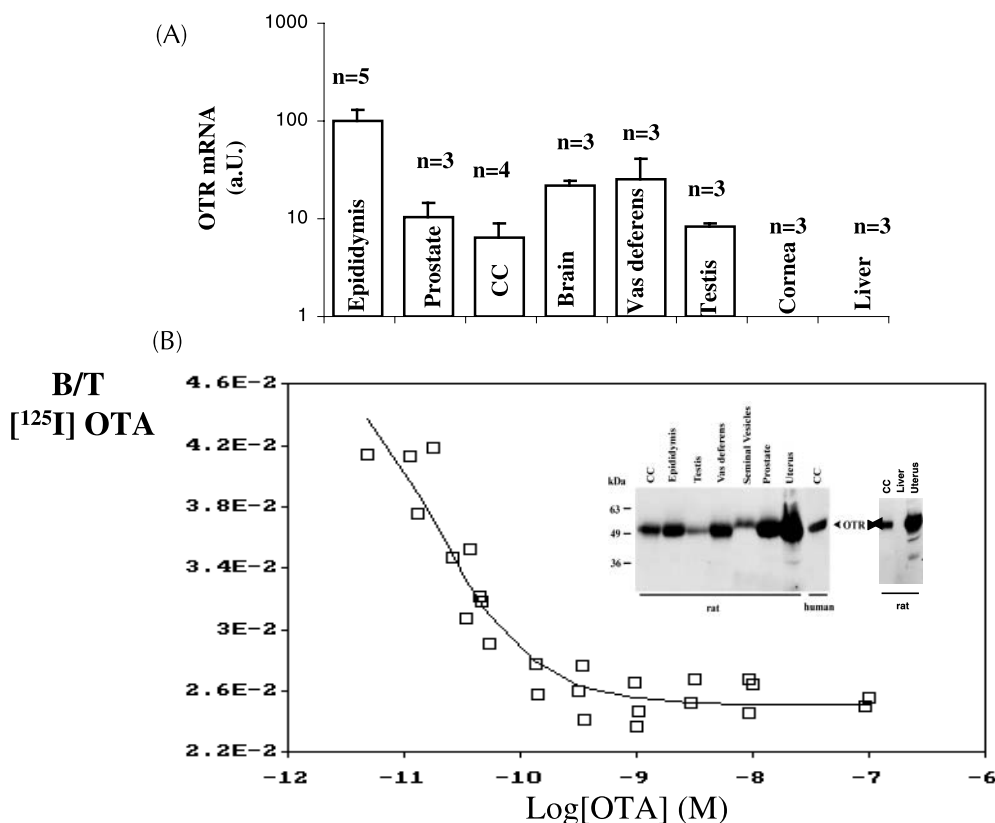
#### Contractility studies

As previously reported for rabbit (Filippi *et al.* 2002b, Vignozzi *et al.* 2004), rat CC strips were attached to an isometric muscle bath system and equilibrated for at least 60 min at 37 °C in Krebs-bicarbonate solution containing

(in mM) NaCl 118.1, KCl 4.7, KH<sub>2</sub>PO<sub>4</sub> 1.0, MgSO<sub>4</sub> 4.0, NaHCO<sub>3</sub> 25.0, CaCl<sub>2</sub> 2.5 and glucose 11.1 in a bath chamber, gassed with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Tissue resting force was set at 500 mg and changes in isometric tension were recorded on a chart polygraph (Battaglia Rangoni, San Giorgio di Piano, Bologna, Italy). High potassium salt solution (KCl) increased the tonic tension with a maximum effect obtained at 80 mM. This value was taken as 100%, and the increase recorded in the presence of different concentrations of OT (1–10 000 nM) and its analogs referred to this value. Cumulative drug concentrations were added, at 7-min intervals, to the bath to obtain a concentration-dependent curve; a 30-min pre-treatment with a selective antagonist (atosiban) was performed before the concentration-response curve for the agonist.

#### Physiological erection studies

SD rats (280–360 g) were anesthetized with pentobarbital (Abbot, Campoverde d'Aprilie, LT, Italy) 45 mg/kg intraperitoneal injection. Mean arterial pressure (MAP) was continuously monitored via the femoral artery. Both crura were exposed and each was perforated with a 26-gauge needle connected to PE-50 tubing for ICP recording and drug delivery. MAP and ICP were recorded via a pressure transducer (World Precision Instruments Inc, Sarasota, FL, USA) connected to a recorder TA240 (Gold Inc, Cleveland, OH, USA). For ES, a bipolar platinum electrode attached to an ST6 stimulator (Bio-medica Mangoni Inc, Pisa, Italy) was mounted on the cavernous nerve. ES (width 5 ms, duration 30 s, 2.5 V) at different frequencies (1, 2, 4, 8, 16, 32 Hz) was performed, with the maximal ICP obtained at 16 Hz. OT (Fort Dodge Veterinaria, S.A., Girona, Spain) ICI was tested at this frequency at the following doses (in mU) 600, 200, 60, 20, 6 and 2, 15 min after the first ES and 5 min before the second stimulation. Atosiban (45 µg) plus OT (60 mU) was administered according to the previously described protocol, and atosiban was delivered 5 min before OT injection. Papaverine (0.35 mg) was found to elicit the optimal response as reported previously (Chen *et al.* 1992, Martinez-Pineiro *et al.* 1994). The effect of OT and OT plus antagonist was also investigated on the increase in ICP induced by this pharmacostimulation with the only difference from the ES protocol being that there was a 30-min wait between the two responses. No more than two doses were tested for each animal. The effect of the OT antagonist atosiban (45 µg), alone, on erectile response was tested by ES of the cavernous nerve at varying stimulation frequencies (2, 4, 16 Hz). Atosiban was intracavernously injected 10 min after the first ES and 5 min before the second ES. The erectile response elicited by electro- or pharmaco-stimulation were quantified by calculating the ratio of max ICP/MAP × 100. The max ICP is the maximal ICP rise, with MAP being the mean artery pressure during the plateau phase.



**Figure 1** Characterization of OTR in rat CC. (A) Quantitative detection of mRNA for OTR in rat tissues by real-time RT-PCR. Data are expressed as means  $\pm$  S.E.M. of arbitrary units (a.u.) calculated according to the comparative Ct method. (B) Binding studies. Homologous competition curve for <sup>125</sup>I-OTA in CC. Ordinate: B/T, bound to total ratio for <sup>125</sup>I-OTA. Abscissa: total concentration (molar) of labeled+unlabeled OTA. Insert: Western blot detection of OTR in rat reproductive tissues. A single band at the expected molecular mass of 55 kDa is present at different levels in all rat genital tissues analyzed. The human CC and rat uterus were run as positive controls and the rat liver as a negative control. Molecular mass markers (kDa) are indicated to the left of the blot.

### Statistical analysis

Results are expressed as means  $\pm$  S.E.M. for *n* experiments. Statistical analysis was performed with Student's *t*-test followed by Fisher's test when appropriate. *P* < 0.05 was taken as significant. Values of half-maximal response inhibiting concentrations (IC<sub>50</sub>) were calculated by using the ALLFIT program (De Lean *et al.* 1978).

## Results

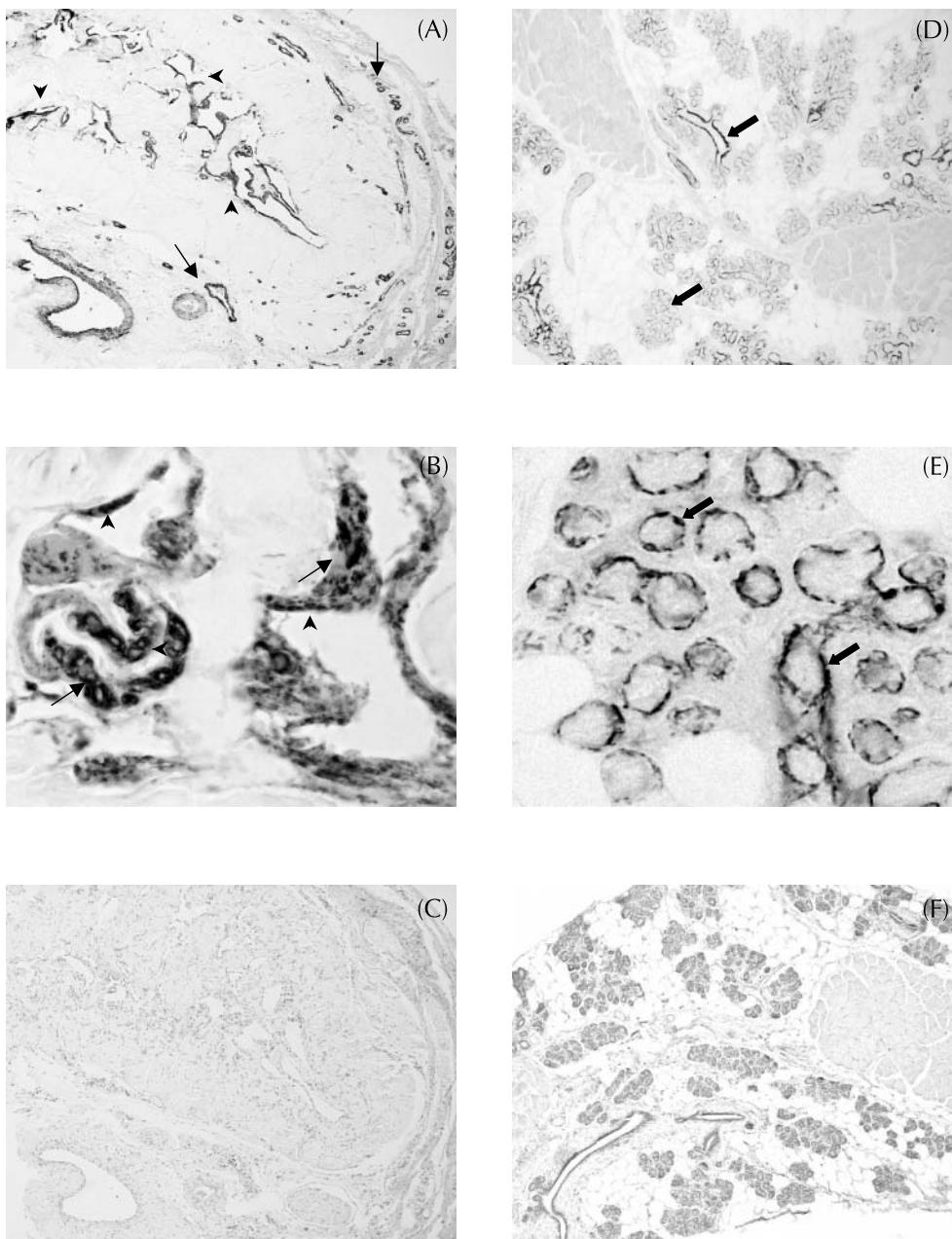
The quantitative distribution of OTR mRNA in the rat male genital tract is shown in Fig. 1A. Specific transcripts for OTR are expressed in all the tissues investigated, including penis. Penile expression of OTR was comparable to that observed in testis and prostate, although one log unit lower than in the epididymis, while the rat cornea and liver, considered as negative controls (Assinder *et al.* 2004), reveal the lowest level of OTR mRNA. To

further characterize OTR in rat CC, we performed binding studies using the OTR selective radiolabeled ligand <sup>125</sup>I-OTA, according to the previously described protocol (Maggi *et al.* 1987, Peri *et al.* 1997, 1998, Vignozzi *et al.* 2004). Figure 1B shows the simultaneous fitting, using the LIGAND program (Munson & Rodbard 1980) of homologous competition curves for <sup>125</sup>I-OTA, derived from three separate experiments. <sup>125</sup>I-OTA binds

**Table 1** Binding parameters derived from the simultaneous fitting (using the LIGAND program) of 3 homologous competition curves for <sup>125</sup>I-OTA performed in corpora cavernosa from 3 independent membrane preparations

Affinity constant, $K_d$ (pM)	$17.0 \pm 6.50$
Binding capacity, $B_{max}$ (fmol/mg protein)	$15.7 \pm 5.00$
Non specific binding (% of total)	$2.0 \pm 0.04$

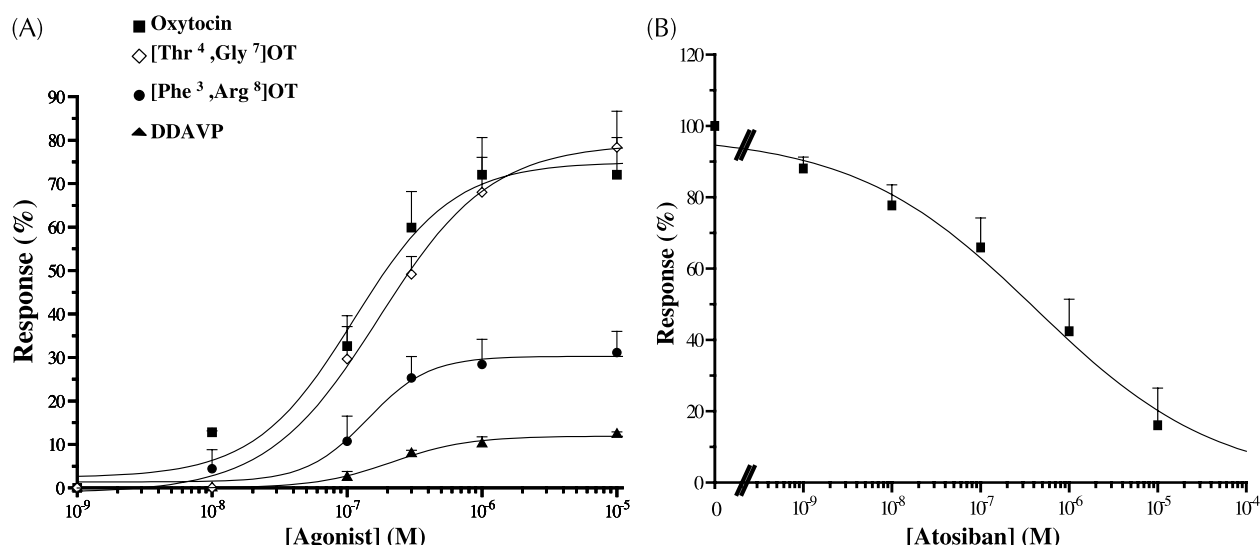




**Figure 2** Immunolocalization of OTR in transverse sections of rat CC (A,B,C) and in rat breast (D,E,F). Panels A (magnification  $\times 40$ ) and B (magnification  $\times 200$ ) show an intense immunopositivity for OTR both in the endothelial (arrows heads) and smooth muscle cells (arrows) of the arteriolar wall and lacunar spaces. Panels D (magnification  $\times 40$ ) and E (magnification  $\times 200$ ) show the presence of positive labeling in myoepithelial cells of ducts and alveoli (black arrows). Immunostaining was obtained with CHINA/1F3 antibody (dilution 1:200). Panels C and F show the control sections obtained by pre-incubating the primary antibodies for OTR with OTR-enriched microsomes (magnification  $\times 40$ ) and are counterstained with hematoxylin.

with high affinity to a homogeneous class of binding sites. These sites are expressed in rat CC at a density similar to that which we have already described for the OTR in

human and rabbit penis (Vignozzi *et al.* 2004). Binding parameters are reported in Table 1. We also performed Western blot analysis of OTR in male rat genital tissues,



**Figure 3** Characterization of OTR in rat CC (contractility studies). (A) Effect of increasing concentrations of OT (solid squares), [Thr<sup>4</sup>,Gly<sup>7</sup>]OT (open diamonds), [Phe<sup>2</sup>,Orn<sup>8</sup>]VT (solid circles) and DDAVP (solid triangles) on the basal contractility of rat CC preparations. Ordinate: increase in basal tone, expressed as a percentage of the maximal response obtained with KCl (80 mM). Abscissa: molar concentration of the agonists. Data are expressed as means  $\pm$  S.E.M. of at least 4 separate experiments. (B) Effect of increasing concentrations of atosiban, an OT antagonist, on the tone induced by a fixed concentration of OT (1  $\mu$ M) in rat CC ( $n=4$ ).

using rat uterus and human CC as positive controls and rat liver as a negative control (Fig. 1 inset). In agreement with previous studies in humans and rabbits (Vignozzi *et al.* 2004), a single band of the expected molecular mass was detected in all tissues examined, including rat CC.

To localize OTR in rat cavernous tissue we performed immunohistochemical studies (Fig. 2A,B,C), using the same antibody as employed for Western blot studies (CHINA/1F3) and compared them with the results using mammary gland (Fig. 2D,E,F), considered as a positive control. An OTR positive staining was observed in the endothelial cells and in the smooth muscle cells of the arteriolar wall and trabecular spaces (Fig. 2A,B).

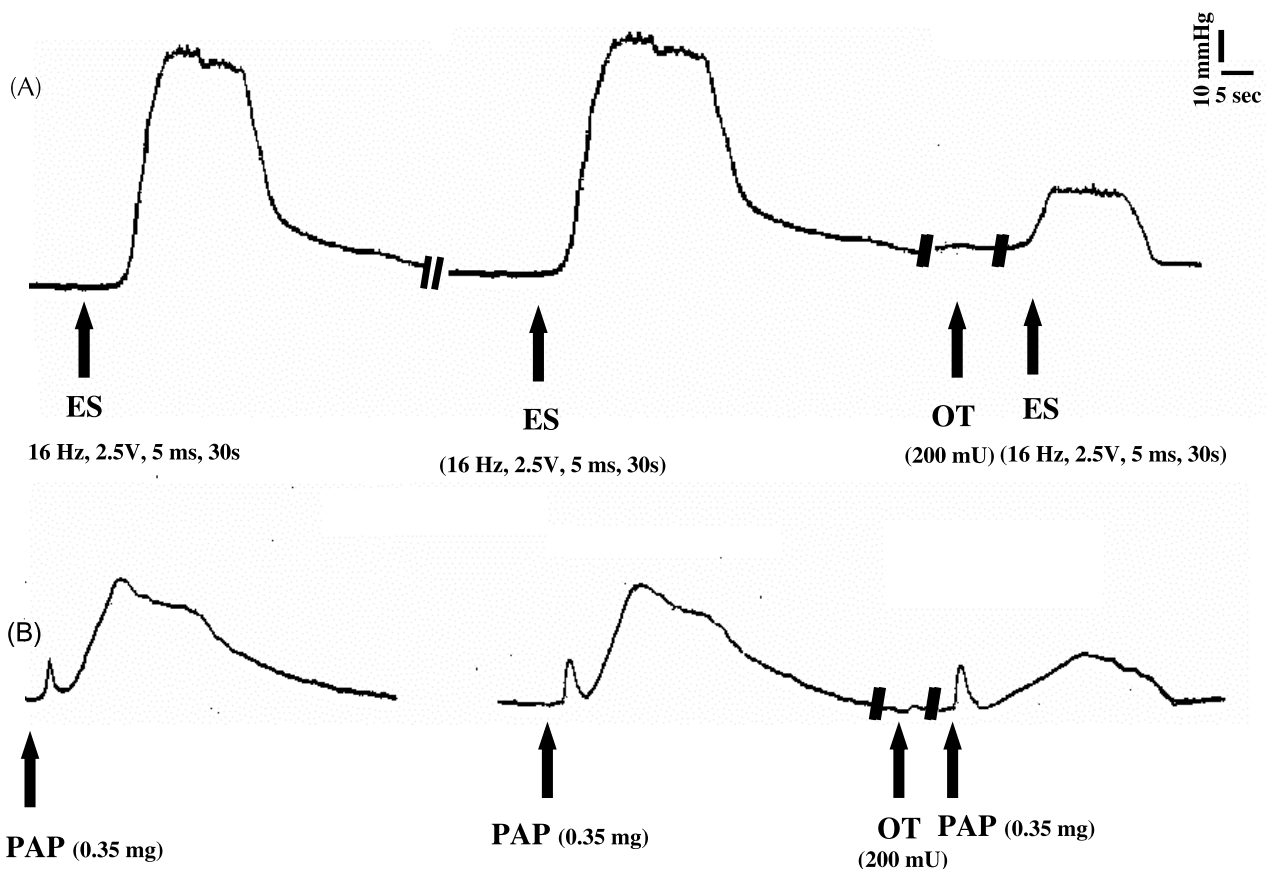
A similar pattern of OTR expression was demonstrated in the mammary gland, where both the epithelial and the surrounding smooth muscle compartment were positively stained (Fig. 2D,E). The specificity of staining was evaluated through the complete absence of labeling obtained by omitting the primary antibody or pre-absorbing it with human myometrial membranes enriched with OTR (Fig. 2C,F), as previously described (Vignozzi *et al.* 2004).

To pharmacologically characterize OTR expression in the rat penis, we performed *in vitro* contractility studies in CC strips using several neurohypophysial hormone analogs showing selectivity for the different receptor subtypes. The OT and the selective OTR agonist [Thr<sup>4</sup>, Gly<sup>7</sup>]OT induced a sustained and virtually identical increase in tension ( $E_{\max}=77.2 \pm 2.8\%$ ), while the selective V<sub>1</sub> and V<sub>2</sub> agonists were definitively less active (Fig. 3A). In addition, increasing the concentration of the

OT antagonist, atosiban, dose-dependently counteracted the contractile effect of 1  $\mu$ M OT (Fig. 3B).

The *in vivo* effect of locally administered OT on physiological erections was also studied. The baseline ICP and MAP, recorded at the beginning of the experiment, were  $10.8 \pm 0.35$  mmHg and  $125.1 \pm 1.6$  mmHg respectively ( $n=60$ ). ES of the cavernous nerve induced a frequency-dependent increase in ICP (data not shown), reaching a maximum ICP ( $68.1 \pm 1.64$  mmHg) at 16 Hz. Papaverine (0.35 mg) ICI also induced a sustained increase in ICP ( $45.6 \pm 1.39$  mmHg). Figure 4 (left side) shows the results from a typical experiment of ES (upper tracing) and papaverine (lower tracing). As shown on the right side of Fig. 4, OT (200 mU) blunted the effect of both stimulators. Figure 5A shows that this inhibitory effect of OT is dose-dependent, with similar IC<sub>50</sub>s for both ES- and papaverine-induced responses (shared IC<sub>50</sub>= $117.7 \pm 37$  mU). The OT antagonist atosiban (45  $\mu$ g intracavernously) completely abrogated the inhibitory effect of OT (60 mU, a dose close to the IC<sub>50</sub>) on both the ES and the papaverine-induced ICP increase (Fig. 5B).

Finally, to evaluate whether blocking endogenous of OT action would increase ES-induced erection, we administered atosiban (45  $\mu$ g) alone by ICI during ES with increasing frequencies (2–16 Hz). The results are shown in Fig. 6. Atosiban significantly increased the mean ICP/MAP ratio at lower stimulation frequencies (2 Hz= $P<0.001$  and 4 Hz= $P<0.05$  vs control,  $n=5$  for each frequency), but not at the maximal frequency (16 Hz). Interestingly, after atosiban ICI, the maximal ICP/MAP



**Figure 4** Representative ICP tracings. Erectile response elicited by electrical stimulation (ES; 16 Hz, 2.5 V, 5 ms, 30 s) of the rat cavernous nerve (upper tracing) and by papaverine (PAP; 0.35 mg) intracavernous injection (lower tracing) is shown. Tracings on the right side demonstrate ICP changes induced by ES (upper tracing) and papaverine (lower tracing) before and after intracavernous administration of 200 mU OT.

ratio induced by 4 Hz stimulation ( $56.3 \pm 1.9$ ) was not different from the value obtained with the maximal (16 Hz) stimulation ( $59.02 \pm 2.1$ ). In unstimulated preparations, atosiban slightly, but not significantly, increased the basal ICP/MAP ratio when compared with control rats (control =  $9.67 \pm 0.96$ , atosiban =  $10.8 \pm 1.65$ ,  $n = 15$ ,  $P = 0.11$ ).

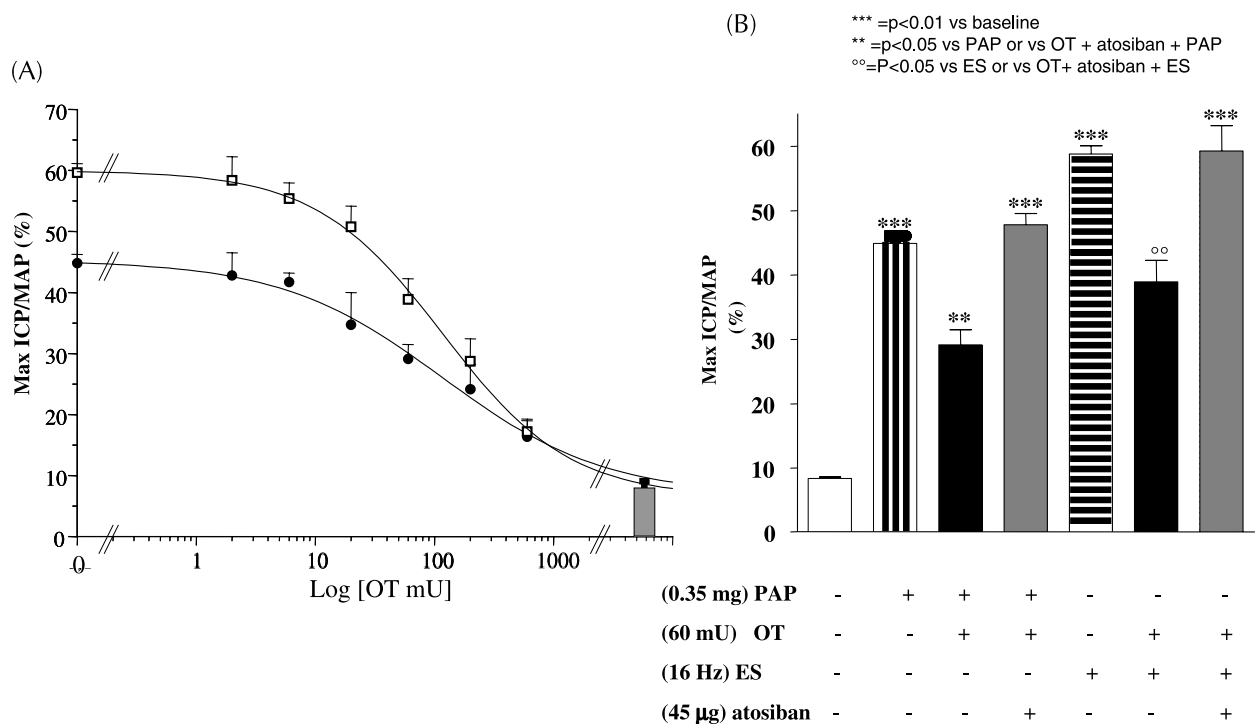
## Discussion

The present study extended a previous observation on the presence of biologically active OTR in the penis of rabbits and humans (Vignozzi *et al.* 2004) to rats. Also it shows, for the first time, that exogenous injection of OTR ligands into rat CC affects penile erection *in vivo*.

To identify, characterize and localize OTR in rat CC, we performed combined real-time RT-PCR, radioligand binding, Western blot, immunohistochemical and *in vitro* contractility studies. Results obtained from all these different methods support the view that OTR is expressed and biologically active in the rat penis as previously

reported by us in the rabbit and human penis (Vignozzi *et al.* 2004). Interestingly, the OTR gene is expressed in the penis in similar concentrations as those found in the prostate and testis, classically considered male targets for OT (Bathgate & Sernia 1994, Einspanier & Ivell 1997, Ivell *et al.* 1997, Frayne & Nicholson 1995, 1998, Assinder *et al.* 2000, 2004, Whittington *et al.* 2001). In the latter tissues, OTR regulates steroidogenesis (Nicholson *et al.* 1991, Nicholson & Jenkin 1995, Frayne & Nicholson 1995) and contractility (Bodanszky *et al.* 1992, Frayne *et al.* 1996, Harris & Nicholson 1998). In the penis, OTR is mainly localized in smooth muscle and regulates its *in vitro* contractile tone.

The main finding of this study is the original demonstration that intracavernous delivering of OT led to a dose-dependent inhibition of the ICP increase elicited by either ES or papaverine. Concomitant administration of the OTR antagonist, atosiban, completely abolished the OT-induced detumescence. Another important finding of this study is that ICI of atosiban facilitated a sub-maximal erectile response induced by ES of the cavernous nerve, at



**Figure 5** Effects of OT on ICP. (A) Increasing doses of OT (2–600 mU) on intracavernous pressure induced by electrical stimulation (ES; 16 Hz, 2.5 V, 5 ms, 30 s, open squares) and papaverine (0.35 mg, solid circles), normalized as a percentage of mean arterial pressure (max ICP/MAP × 100). The ICP rise in the absence of OT injection is taken as control. The baseline ICP/MAP (%) value (grey column) is also provided. Values are expressed as means ± S.E.M. of at least 4 separate rats for each dose. (B) Effect of OT (60 mU) with (grey columns) or without (solid columns) the OT antagonist atosiban (45 µg) on ICP increase (max ICP/MAP × 100) induced by ES (16 Hz, 2.5 V, 5 ms, 30 s) or papaverine (0.35 mg) ICI. The percentage of max ICP/MAP increases induced by ES (horizontally hatched columns) and by papaverine (PAP, vertically hatched columns) before OT injection are taken as control. The baseline ICP/MAP (%) value (open column) is also represented. Values are shown as means ± S.E.M. of at least 4 separate experiments. \*\*\*P<0.01 vs baseline; \*\*P<0.05 vs either PAP or OT+atosiban+PAP; °°P<0.05 vs either ES or OT+atosiban+ES.

lower stimulation frequencies. This supports the view that OTR mediates penile detumescence *in vivo* and suggests that endogenous OT may exert a tonic role in maintaining penile flaccidity, which can be unmasked by the pharmacological block of its receptor. If these *in vivo* findings in the rat model are confirmed also in humans, we hypothesize that intracavernous injection of OT could be clinically helpful in treating low-flow priapism, as an alternative therapy to adrenergic agonists which are not completely devoid of dangerous systemic side-effects. In addition, the use of atosiban to increase the erectile response to other vasorelaxant stimuli might be envisaged.

Although it is unclear which is the physiological source of penile OT, it is interesting to note that the affinity constants of the OTR which we identified in the penis in this ( $K_d=17$  pM) as well as in a previous study (Vignozzi *et al.* 2004) are compatible with the circulating concentration of OT. Interestingly, circulating OT substantially increases during male orgasm (Ogawa *et al.* 1980, Carmichael *et al.* 1987, Murphy *et al.* 1987, Uckert *et al.* 2003). Orgasm and semen ejaculation are usually accompanied by a prompt detumescence, which

characterizes the post-ejaculatory refractory period. Hence, it is possible that OT plays a physiological role in mediating post-orgasmic penile detumescence. However, it is also possible that OT is generated locally in the penile tissue, as has been described for other male genital tissues such as testis (Nicholson *et al.* 1984, 1986, Foo *et al.* 1991, Nicholson & Hardy 1992, Einspanier & Ivell 1997), epididymis (Harris *et al.* 1996, Assinder *et al.* 2000) and prostate (Gemmell & Sernia 1989, Nicholson 1996, Jenkin & Nicholson 1999, Assinder *et al.* 2004, Whittington *et al.* 2004), exerting an autocrine/paracrine role in regulating cavernous smooth muscle tone.

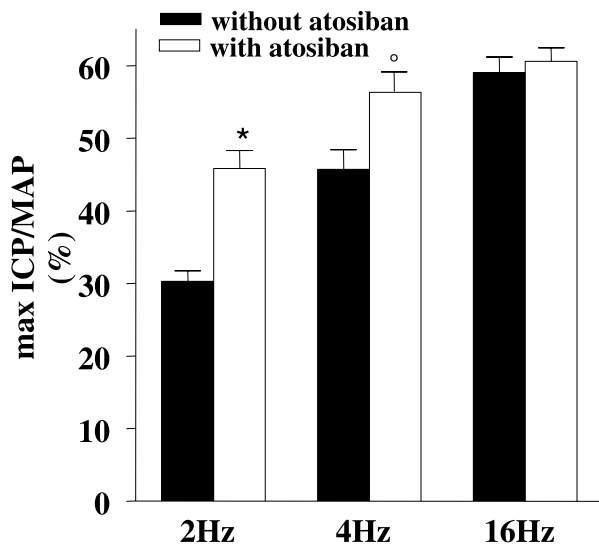
In conclusion, we have demonstrated for the first time that OTR exists in rat CC and mediates contractility *in vitro* and *in vivo*, suggesting a possible role for this receptor in regulating penile erection.

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\*  $p < 0.001$  and ° =  $p < 0.05$  vs control



**Figure 6** Effects of the OT antagonist atosiban, alone, on erectile response. The ICP increase was induced by electrical stimulation (ES; 2.5 V, 5 ms, 30 s) at varying stimulation frequencies (2, 4, 16 Hz). Atosiban (45 µg) was intracavernously injected 10 min after the first ES and 5 min before the second ES. The first response (before atosiban injection) is taken as control (solid column). Values were normalized as a percentage of the mean arterial pressure (max ICP/MAP × 100) and are shown as means ± S.E.M. (\* $P < 0.001$  and ° $P < 0.05$  vs control,  $n = 5$  for each frequency).

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