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RESEARCH PAPER

Oxytocin-induced contractions within rat and rabbit ejaculatory tissues are mediated by vasopressin V_{1A} receptors and not oxytocin receptors

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Background and purpose: Oxytocin is believed to be involved in ejaculation by increasing sperm number and contracting ejaculatory tissues. However, oxytocin may mediate these effects via oxytocin or vasopressin (AVP) receptors. The aim of this study was to determine the effect of oxytocin and AVP on peripheral tissues involved in ejaculation and to identify the receptor subtype(s) involved.
Experimental approach: Standard tissue bath techniques were used to measure isometric tension from tissues involved in ejaculation and erection.
Key results: Oxytocin and AVP failed to elicit a tonic contractile response in rat and rabbit testes, vas deferens, epididymis, seminal vesicles and prostate. In contrast, oxytocin and AVP elicited large tonic contractions in erectile (corpus spongiosum and corpus cavernosum) and ejaculatory (prostatic urethra, bladder neck and ejaculatory duct) tissues in a concentration-dependent manner. The selective oxytocin agonist, [Thr^4,Gly^7]-oxytocin and the V_2 agonist, [deamino-Cys^1,Val^4,D-Arg^8]-vasopressin (dDAVP), failed to contract tissues. Oxytocin and AVP-induced contractions were weakly antagonized by the selective oxytocin antagonist, L-368899 but potently antagonized by the V_{1A} antagonist, SR49059. The V_{1B} antagonist SSR149415 failed to antagonize AVP contractions except in rabbit bladder neck. Neither L-368899 nor SR49059 antagonized endothelin-1-induced contractions.
Conclusions and implications: The contractile effect of oxytocin on rat and rabbit ejaculatory and erectile tissues is mediated via V_{1A} receptors. Endothelin-1-induced contractions are not due to endogenous oxytocin or AVP release. V_{1A} receptor antagonists may have a therapeutic role in both erectile dysfunction and premature ejaculation.
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Keywords: oxytocin; vasopressin; V_{1A}; ejaculation; contraction; *in vitro*

Abbreviations: AVP, arginine vasopressin; dDAVP, [deamino-Cys^1,Val^4,D-Arg^8]-vasopressin; ET-1, endothelin-1; TGOT, [Thr^4,Gly^7]-oxyctocin

Introduction

Premature ejaculation is the most common sexual disorder in men (see Mulhall, 2006). Ejaculation is a two-phase phenomenon involving emission and expulsion of semen. The physiological events underlying the first emission phase includes the peristaltic contraction of the epididymis, contraction of the seminal vesicles, prostate and bladder neck with propulsion of seminal fluid into the posterior urethra. The second expulsion phase involves the rhythmic contraction of striated pelvic floor muscles and bladder neck closure. In humans, the external urinary sphincter relaxes during the expulsion phase whereas in rats it contracts.

Ejaculation has been proposed to be under the control of neurohypophyseal hormones. Oxytocin is believed to be the main hormone-facilitating semen emission and has been the centre of interest in ejaculation for over 40 years. Plasma oxytocin levels increase around the time of ejaculation in rams (Sharma and Hays, 1973), bulls (Peeters *et al.*, 1983), rabbit (Stoneham *et al.*, 1985) and man (Ogawa *et al.*, 1980; Murphy *et al.*, 1987). The oxytocin rise during ejaculation may be important for spermatogenesis as oxytocin has been shown peripherally to increase sperm volume and concentration in ejaculate in a number of species (Bereznev, 1963; Kihlstrom and Melin, 1963; Fjellstrom *et al.*, 1968; Levin, 1968; Knight and Lindsay, 1970; Voglmayr, 1975; Sharma and Hays, 1976; Ågmo *et al.*, 1978; Berndtson and Igboeli, 1988; Frayne *et al.*, 1996). In man, oxytocin doubles the number of ejaculated sperms from oligozoospermic patients (Filippi *et al.*, 2002b).

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A second hypothesis for the rise in oxytocin levels around the time of ejaculation is that oxytocin has a physiological role in modulating contractile activity throughout the male ejaculatory tract hence aiding sperm transport. Oxytocin has been shown both *in vivo* and *in vitro* to increase contractility of the epididymis, vas deferens and prostate from human, rabbit, mouse and rat (Hib, 1974a, b, 1977; Knight, 1974b; Jaakkola and Talo, 1981; Bodanszky *et al*., 1992; Filippi *et al*., 2002a, b, 2005; Studdard *et al*., 2002) and hence maybe involved in ejaculation (Melin, 1970). The presence of oxytocin receptors throughout the reproductive tract supports a peripheral site of action for oxytocin (Maggi *et al*., 1987; Bathgate and Sernia, 1994; Einspanier and Ivell, 1997; Frayne and Nicholson, 1998; Whittington *et al*., 2001; Filippi *et al*., 2002a, b; Zhang *et al*., 2005). However, these studies showed that the oxytocin receptor is mainly present on epithelial cells of the epididymis, Sertoli and Leydig cells of the testes and stromal cells of prostate with the exception of smooth muscle in the distal epididymis and erectile tissue, implying that oxytocin's main role is in steroidogenesis rather than contractility. In addition, as most studies have used peptide antagonists, which also act at vasopressin (arginine vasopressin; AVP) V_{1A} receptors, no studies have been done using selective non-peptide oxytocin antagonists to show conclusively that oxytocin is acting through oxytocin receptors.

Therefore, the aims of the study were to (a) investigate the effect of oxytocin and vasopressin (AVP) on smooth muscle from all peripheral tissues involved in ejaculation, (b) compare potency and efficacy effects in both rat and rabbit and (c) use selective non-peptide oxytocin and V_{1A} receptor antagonists to identify the receptor subtype(s) mediating the effects of oxytocin and AVP in these tissues.

Methods

All animal procedures and experiments were carried out in compliance with the UK legislation and in strict accordance with the Animals (Scientific Procedures) Act 1986. Harlan New Zealand white rabbits (1–3 kg, 9–18 weeks) obtained from Charles River were killed by an overdose of pentobarbital sodium (Pentoject; Animalcare, York, UK) ($50\,mg\,kg^{-1}$), Sprague–Dawley rats (250–350 g, 56–63 days) also obtained from Charles River were killed by a CO_2 overdose. The whole urogenital tract was removed and immersed into Krebs solution (composition (mM) 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 25 $NaHCO_3$, 1.2 $MgSO_4$, 1.2 KH_2PO_4 and 11 glucose) continuously aerated with 95% O_2/5% CO_2. Ejaculatory tissues (1–2 cm in length) were dissected and then mounted longitudinally, using cotton sutures into 16 channel 5 mL organ baths (Pfizer, Sandwich, UK). One end of the tissue was attached to the bottom of the bath and the other to a Thames side transducer (Maywood Ltd) under 1 g tension (2 g for rat corpus spongiosum and rabbit corpus cavernosum). Each bath was perfused with Krebs solution maintained at 37 °C at a constant flow rate of $6\,mL\,min^{-1}$. Each bath in addition to the perfusion of Krebs had an independent carbogen (95% O_2/5% CO_2) supply allowing individual tissues to be continually aerated and equilibrated for 1 h. Tissue responses were acquired and analysed using Notocord HEM software.

Drug-induced contractions

A baseline recording was taken following which agonists were applied cumulatively to all tissues, with each concentration reaching a plateau prior to the next addition. Antagonists or vehicle control were applied 30 min prior to the agonist concentration–response curve. The bath perfusion was stopped 10–15 min prior to drug application to allow the baseline to stabilize. The antagonists used were L-368899, SR49059 and SSR149415. L-368899 is a potent selective oxytocin antagonist with 50-fold selectivity over V_{1A} and V_2 receptors (Williams *et al*., 1994). Likewise, SR49059 is a potent V_{1A} antagonist that has >50-fold selectivity over oxytocin, V_{1B} and V_2 receptors in several species (Serradeil-Le Gal *et al*., 1993). SSR149415 is a selective V_{1B} antagonist with >100-fold potency over other AVP and oxytocin receptors (Serradeil-Le Gal *et al*., 2002).

Analysis

Data have been normalized as (effect–baseline) × 100 and expressed as mean ± standard error of the mean (s.e.mean). EC_{50} values were calculated as the molar concentration at which oxytocin and AVP produced 50% of the maximum response. Experimental data were analysed to obtain pK_B values using the global nonlinear regression method described by Lew and Angus (1995) were three concentrations of antagonists were used. Advantages offered by this method over the traditional Schild method have been discussed previously (Lew and Angus, 1995) but does not require the within-tissue control concentration–response curves required for standard Schild regression methods. Reproducible concentration–response curves to oxytocin and AVP were not obtained in all the ejaculatory tissues studied. Also as the method uses the pEC_{50} values directly and does not depend on the concentration ratios, the pEC_{50} for the control curves enters the analysis as any other pEC_{50} and the heavy dependence upon the control curve data that occurs with Schild analysis is avoided. The Lew and Angus model can be expressed as $pEC_{50} = -\log_{10}([B] + 10^{-pK_B}) - (pK_B - pEC_{50|B=0})$ where [B] is the antagonist concentration. As the oxytocin and the V_{1A} antagonist used are reported to be competitive antagonists (L-368899 Pettibone *et al*., 2004; SR49059 Serradeil-Le Gal *et al*., 1993) where only a single or two high concentrations of antagonist were effective, the Gaddum equation ($pK_B = \log$ (concentration ratio − 1) − log (antagonist concentration)) was used (Gaddum, 1957; http://www.pdg.cnb.uam.es/cursos/BioInfo2002/pages/Farmac/Comput_Lab/Guia_Glaxo/chap2d.html#pkb). Figures displaying three concentration antagonist concentrations have used the Lew and Angus equation, whereas figures displaying one or two selective concentrations of antagonist have used the Gaddum equation to predict pK_B values. *n* represents the number of animals.

Materials

Oxytocin, AVP, TGOT ([Thr^4,Gly^7]-oxyctocin), dDAVP ([deamino-Cys^1,Val^4,D-Arg^8]-vasopressin), endothelin-1 (ET-1)

and noradrenaline were obtained from Sigma-Aldrich Company Ltd, (Dorset, UK). L-368899, SR49059 and SSR149415 were synthesized by Pfizer as described in the literature (Serradeil-Le Gal *et al*., 1993; Thompson *et al*., 1997; Serradeil-Le Gal *et al*., 2002). All drugs were dissolved in distilled water except for L-368899 and SR49059 that were dissolved in dimethyl sulphoxide with a final bath concentration of $\leqslant$0.1% dimethyl sulphoxide. Vehicle-matched controls showed no dimethyl sulphoxide effects up to 0.1%. All drug solutions were made up freshly, on the day of the experiment.

Results

Effect of oxytocin on rat and rabbit ejaculatory tissues in vitro
Rat and rabbit ejaculatory and erectile smooth muscle tissues were assessed. In the rat, the ejaculatory duct and ampulla were too small to use. Oxytocin (10 μM) failed to elicit a tonic contractile response in testes ($n=4$; $P>0.05$), epididymis ($n=4$; $P>0.05$), vas deferens ($n=4$; $P>0.05$), seminal vesicles ($n=8$; $P>0.05$) and prostate ($n=4$; $P>0.05$). As a positive control, all tissues contracted to noradrenaline (10 μM; >100 mg) implying all tissues were viable (Table 1). In contrast, oxytocin elicited a large tonic contractile response in a concentration-dependent manner within the corpus spongiosum, prostatic urethra and bladder neck (Figure 1a; Table 2). As shown in Table 2, the potency of oxytocin against each of the preparations was not high but had a slope close to unity.

In the rabbit ejaculatory tract, oxytocin (10 μM) also failed to contract the testes ($n=4$; $P>0.05$), epididymis ($n=6$; $P>0.05$), vas deferens ($n=6$; $P>0.05$), seminal vesicles ($n=22$; $P>0.05$), as well as the ampulla ($n=8$; $P>0.05$). All tissues contracted to noradrenaline (10 μM; Table 1). Oxytocin-induced concentration-dependent contractions in the rabbit corpus cavernosum, prostatic urethra, bladder neck and ejaculatory duct (Figure 1b; Table 2). The ejaculatory duct is one of two tubes, each formed by union of the ampulla of the vas deferens and the excretory duct of a seminal vesicle. The ejaculatory duct opens into the urethra halfway through the prostate gland. In rabbit and rat tissues, the data from repeated oxytocin concentration–response curves were not reproducible following a 1-h wash therefore only one curve per tissue was constructed.

The selective oxytocin agonist TGOT, however, failed to elicit reproducible concentration–response curves in all ejaculatory tissues. TGOT at high concentrations did elicit a contraction in the erectile corpus spongiosum (10 μM, E_{max} 519 ± 380 mg, $n=4$; EC_{50} 0.70 ± 2.76 nM slope 0.7, $n=4$; data not shown).

Table 1 Responses to noradrenaline (10 μM) in ejaculatory tissues that failed to respond to oxytocin

Species	*Tissue*	*Contraction amplitude (mg)*	n
Rat	Testes	412 ± 95	4/4
	Epididymis	486 ± 38	4/4
	Vas deferens	501 ± 83	4/4
	Seminal vesicles	923 ± 213	4/4
	Prostate	306 ± 52	4/4
Rabbit	Testes	205 ± 37	4/4
	Epididymis	513 ± 85	4/4
	Vas deferens	561 ± 278	4/4
	Seminal vesicles	1518 ± 705	4/4
	Ampulla	894 ± 201	4/4

Noradrenaline responses in rat and rabbit erectile and ejaculatory tissues are shown as mean (± s.e.mean) of the contraction amplitude (mg) and '*n*' refers to the number of tissues responding out of the number of tissues tested.

Which receptor subtype(s) underlie the oxytocin-induced contractions?
To determine whether oxytocin was acting via oxytocin receptors in rat ejaculatory tissues, the potent oxytocin antagonist L-368899 (300 nM–3 μM) was used (rat $K_i=3.6$ nM; Thompson *et al*., 1997). L-368899 (300 nM–3 μM) did not potently antagonize the oxytocin concentration–response curve in the rat corpus spongiosum ($n=3$–4) and prostatic urethra ($n=4$) with a pK_B of 6.89 (slope 0.87) and 6.79 (slope 0.69), respectively, and rat bladder neck ($n=3$–4) with a pK_B of 5.5 (slope 1) (Figure 2a). Similarly to rat tissues, L-368899 did not potently antagonize oxytocin responses in rabbit corpus cavernosum ($n=5$, pK_B 8.02, slope 1), prostatic urethra ($n=5$, pK_B 7.01, slope 1), ejaculatory duct ($n=4$, pK_B 6.34, slope 1) and bladder neck ($n=5$, pK_B 5.89, slope 1) (Figure 3a).

As L-368899 did not potently antagonize the oxytocin contractions in the rat and rabbit ejaculatory tissues, it was possible that oxytocin was acting through the V_{1A} receptor. The selective non-peptide V_{1A} antagonist SR49059 (1–30 nM; rat $K_i=1.6$; Serradeil-Le Gal *et al*., 1993) potently antagonized the oxytocin-induced contractions in both rat and rabbit tissues (pK_B 9.67, slope 0.87 rat corpus spongiosum; pK_B 9.62, slope 0.85 rat prostatic urethra; pK_B 8.68, slope 1 rat bladder neck; pK_B 9.95, slope 1 rabbit corpus cavernosum; pK_B 9.73, slope 1, rabbit prostatic urethra; pK_B 9.19, slope 1, rabbit ejaculatory duct; pK_B 9.81, slope 1 rabbit bladder neck; $n=5$–7, Figures 2b and 3b).

Effect of AVP on rat and rabbit ejaculatory tissues in vitro
As the oxytocin response was potently antagonized by the V_{1A} antagonist, AVP was tested in all smooth muscle preparations shown to elicit oxytocin-induced contractions. Similar to oxytocin, AVP caused concentration-dependent contractions in rat corpus spongiosum, bladder neck and prostatic urethra (Table 3; Figure 4a), whereas in rabbit tissues, AVP also contracted corpus cavernosum, prostatic urethra, bladder neck and ejaculatory duct (Table 3; Figure 5). AVP was more potent in all tissues compared with oxytocin as summarized in Table 3. AVP evoked large spontaneous phasic contractions on smaller tonic contractions in rat prostatic urethra so no further studies were performed with antagonists as it was not possible to quantify potencies accurately.

Arginine vasopressin was competitively but not potently antagonized by high concentrations of L-368899 (3 μM) in rat corpus spongiosum ($n=3$) and bladder neck ($n=11$) with a pK_B of 5.97 and 6.11, respectively (Figure 5a). However, AVP

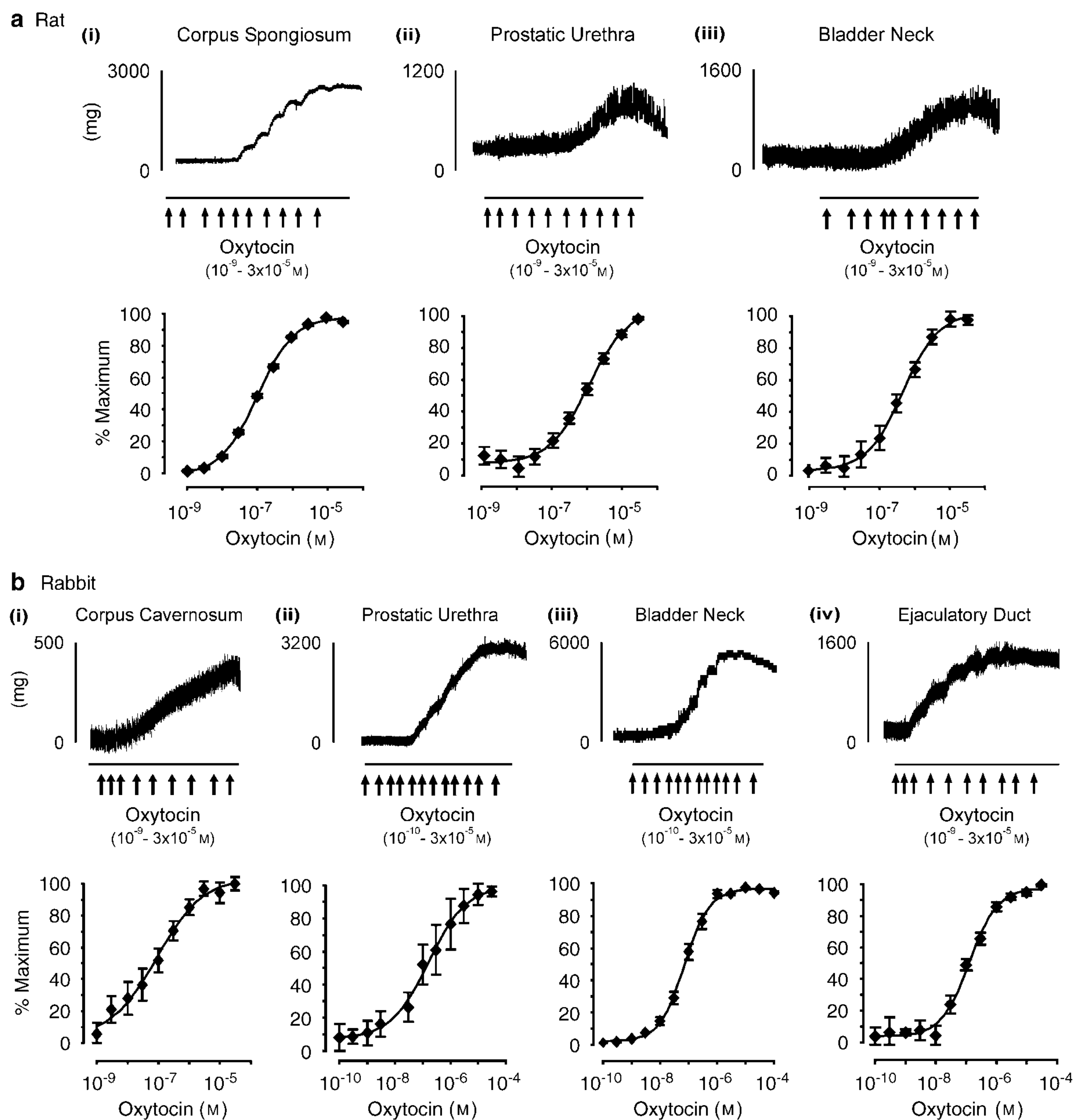

Figure 1 Effect of oxytocin on rat and rabbit reproductive tissues *in vitro*. Top panel showing typical examples of oxytocin in (**a**) rat and (**b**) rabbit (i) corpus spongiosum/cavernosum, (ii) prostatic urethra (iii) bladder neck and (iv) ejaculatory duct. Graphs in (**a**) and (**b**) show the mean (±s.e.mean) effect of oxytocin in each tissue.

Table 2 Comparison of the contractile effects of oxytocin on ejaculatory and erectile smooth muscle from rat and rabbit tissues

Species	*Tissue*	*EC_{50} (nM)*	*Slope*	*E_{max} (mg)*	n
Rat	Corpus spongiosum	105±8.2	0.83±0.04	1262±121	44
	Prostatic urethra	1164±167	0.77±0.07	408±77	37
	Bladder neck	578±219	0.86±0.08	878±74	9
Rabbit	Corpus cavernosum	92±30	0.58±0.13	339±96	20
	Ejaculatory duct	162±29	0.63±0.08	599±168	8
	Prostatic urethra	202±36	0.94±0.14	1048±285	12
	Bladder neck	72±8.8	1.00±0.1	582±237	5

Responses to oxytocin are shown as the mean (±s.e.mean) of the EC_{50} response, slope and E_{max} (mg) together with the number of tissues used for rat and rabbit erectile and ejaculatory tissues.

was potently antagonized by low concentrations of SR49059 (10 nM) in rat corpus spongiosum pK_B 8.81, slope 1 ($n=3$) and bladder neck 8.48, slope 1 ($n=3$) as shown in Figure 5b.

L-368899 (3 μM) also weakly but competitively antagonized AVP responses in rabbit corpus cavernosum ($n=3$; pK_B 6.68, slope 1), bladder neck ($n=4$; pK_B 6.09, slope 1) and ejaculatory duct ($n=4$; pK_B 6.22, slope 1). No pK_B could be determined for the rabbit prostatic urethra. AVP was conclusively shown to act through the V_{1A} receptor as SR49059 (10 nM) potently antagonized the AVP-induced contraction in rabbit corpus cavernosum pK_B 9.50, slope 1 ($n=5$), bladder neck pK_B 9.36, slope 1 ($n=5$), prostatic

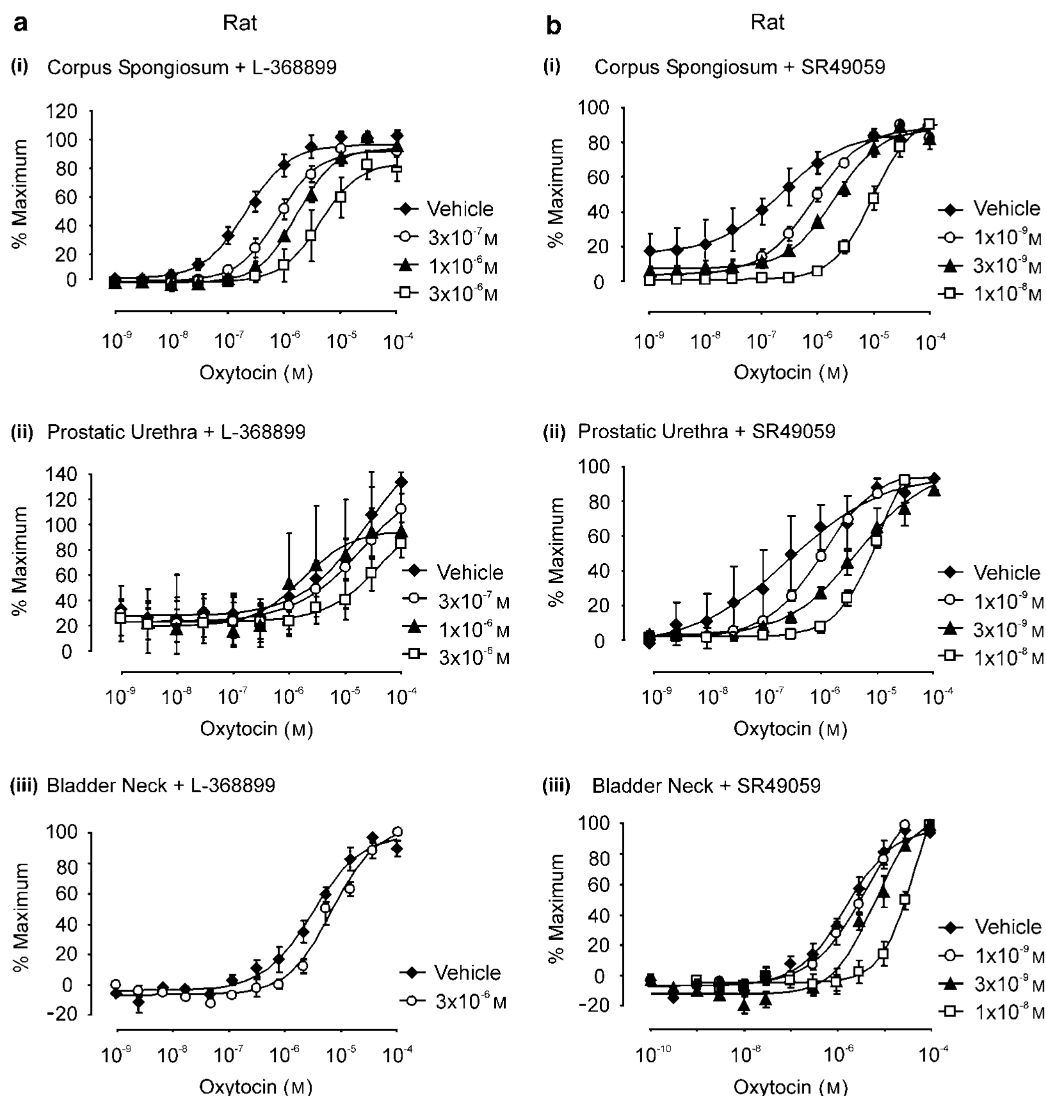

Figure 2 Effect of oxytocin in the presence of the oxytocin antagonist L-368899 and the V_{1A} antagonist SR49059 on rat reproductive tissues. Oxytocin concentration–response curves in rat (i) corpus spongiosum, (ii) prostatic urethra and (iii) bladder neck tissues in the absence (vehicle) or presence of (**a**) L-368899 or (**b**) SR49059.

urethra pK_B 8.86 ($n=5$) and ejaculatory duct pK_B 8.38, slope 1 ($n=3$) (Figure 6).

The V_{1B} antagonist SSR149415 failed to antagonize AVP contractions in rat and rabbit ejaculatory tissues except in rabbit bladder neck (pK_B 9.1; $n=3$). In addition, there was no contractile response observed in any ejaculatory tissue with the selective V_2 agonist dDAVP (1 μM, $n=4$; $P>0.05$).

Effect of an oxytocin and V_{1A} receptor antagonist on ET-1-induced contractions in rat ejaculatory tissues

It has recently been shown that ET-1-induced contractions in the epididymis are mediated through oxytocin receptors (Filippi *et al.*, 2005). In the present study, ET-1-mediated contractions were elicited in the rat corpus spongiosum, prostatic urethra, vas deferens, seminal vesicle and testes (see Table 4). Pretreatment of tissues with L-368899 (100 nM) or SR49059 (10 nM) failed to shift the ET-1 concentration–response curve in any of the tissues.

Discussion

Important findings within the present study are (a) oxytocin failed to induce a tonic contraction in rat and rabbit vas deferens, testes, seminal vesicles and epididymis, (b) oxytocin and AVP induced tonic contractions in corpus spongiosum, corpus cavernosum, prostatic urethra, bladder neck and ejaculatory duct, (c) contractile responses to oxytocin and AVP are more potent in rabbit tissues than the same ejaculatory tissues in rat, (d) oxytocin and AVP-induced contractile effects are mediated through V_{1A} receptors and not oxytocin receptors, (e) the V_{1A} antagonist SR49059 is more potent in rabbit than rat and (f) neither oxytocin nor AVP is involved in ET-1 pro-contractile activity.

For over four decades, it has been known that oxytocin (i.v.) can induce contraction of the seminiferous tubules, epididymis and vas deferens in rat, rabbits and rams *in vivo* (Niemi and Kormano, 1965; Melin, 1970; Knight, 1974b; Hib, 1977). As a result, research has focused on testing the

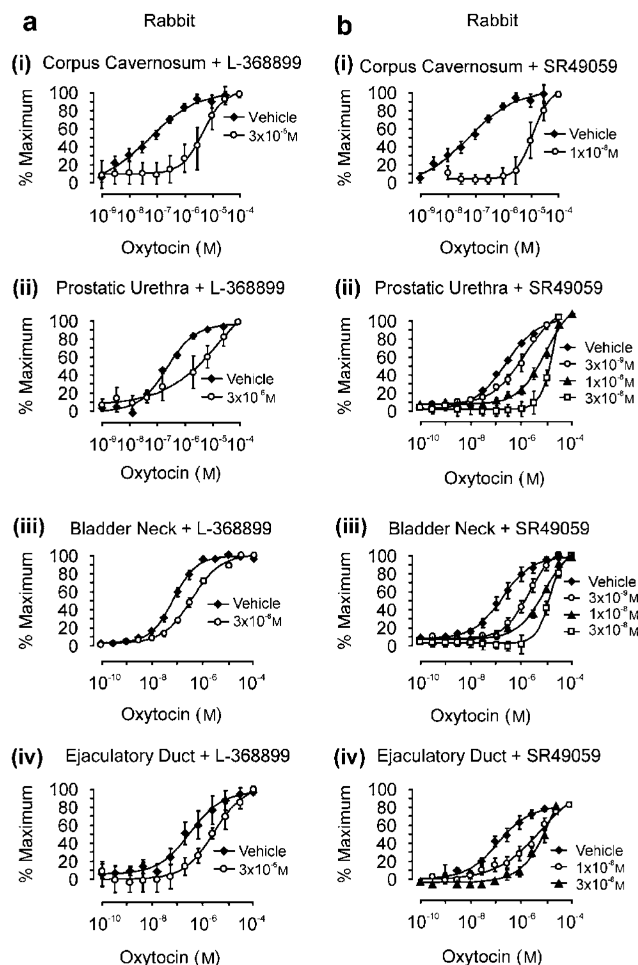

Figure 3 Effect of oxytocin in the presence of the oxytocin antagonist L-368899 and the V_{1A} antagonist SR49059 on rabbit reproductive tissues. Oxytocin concentration–response curves in rabbit (i) corpus cavernosum, (ii) prostatic urethra, (iii) bladder neck and (iv) ejaculatory duct tissues in the absence (vehicle) or presence of (**a**) L-368899 (3×10^{-6} M) or (**b**) varying concentrations of SR49059.

Table 3 Comparison of the contractile effects to AVP on ejaculatory and erectile smooth muscle from rat and rabbit tissues

Species	*Tissue*	*EC_{50} (nM)*	*Slope*	*E_{max} (mg)*	n
Rat	Corpus spongiosum	2.7 ± 1.5	0.6 ± 0.3	1914 ± 265	8
	Prostatic urethra	2.4 ± 1.1	0.5 ± 0.1	858 ± 266	5
	Bladder neck	28 ± 7.5	1.9 ± 0.9	938 ± 167	10
Rabbit	Corpus cavernosum	1.9 ± 0.5	0.8 ± 0.1	402 ± 73	16
	Ejaculatory duct	4.9 ± 1.1	0.9 ± 0.2	188 ± 97	5
	Prostatic urethra	2.7 ± 1.5	0.6 ± 0.2	461 ± 147	4
	Bladder neck	2.6 ± 0.5	0.7 ± 0.1	3141 ± 971	3

Abbreviation: AVP, arginine vasopressin.
Responses to AVP are shown as the mean (± s.e.mean) of the EC_{50} response, slope and E_{max} (mg) together with the number of tissues used for rat and rabbit erectile and ejaculatory tissues.

hypothesis that oxytocin can directly contract mouse, rat and rabbit epididymis *in vitro* (Hib, 1974a, b; Studdard *et al.*, 2002; Filippi *et al.*, 2002a). However, controversial evidence suggests that oxytocin alone is unable to contract ejaculatory tissues *in vitro* such as isolated vasa deferentia or seminal vesicle (Beneit *et al.*, 1980; Sharif and Gokhale, 1986) but has the ability to potentiate (Sharif and Gokhale, 1986) or attenuate noradrenaline-induced contractions (Beneit *et al.*, 1980). Controversy in the literature may reflect the differences in the type of contractions being measured that is phasic spontaneous contractions versus tonic contractions. In the present study, oxytocin alone failed to elicit a tonic contraction in the epididymis, vas deferens, testes, seminal vesicles, ampulla and prostate. For the first time, oxytocin has been shown to elicit large contractions in other peripheral tissues involved in ejaculation, such as the ejaculatory duct, prostatic urethra and bladder neck. In addition to ejaculatory tissues, oxytocin has been demonstrated to contract erectile tissues such as the corpus cavernosum (Tarhan *et al.*, 1995; Vignozzi *et al.*, 2004; Zhang *et al.*, 2005). This finding was confirmed and also supported by contractions mediated within the rat corpus spongiosum implying that oxytocin has the ability to maintain penile flaccidity potentially post ejaculation once local endogenous oxytocin levels increase.

All oxytocin-responsive ejaculatory and erectile tissues responded more potently to AVP raising the question of the receptor subtype(s) involved. Oxytocin is also well known to activate the V_{1A} receptor. Previous studies have attempted to use non-selective peptide antagonists such as the oxytocin antagonist, atosiban (Filippi *et al.*, 2002a; Vignozzi *et al.*, 2004; Zhang *et al.*, 2005) to block oxytocin-induced contractions but selective non-peptide antagonists have not been investigated. In the present study, the selective oxytocin antagonist L-368899 shifted the oxytocin and AVP concentration–response curves only at a concentration more than 300 times its binding K_i value, whereas the selective V_{1A} antagonist SR49059 potently antagonized all contractions at 1–10 times the predicted binding K_i. Together, these data show functionally that the oxytocin and AVP-induced contractions are mediated by the V_{1A} receptor rather than the oxytocin receptor.

In general, although the maximum contraction elicited by oxytocin and AVP were similar, both hormones were about 10-fold more potent in the rabbit compared with equivalent rat ejaculatory tissues. There was no difference in potency in rat and rabbit erectile tissues. Likewise, the V_{1A} antagonist was also more potent in rabbit ejaculatory tissues tested but equipotent in erectile tissues from both rat and rabbit. Species differences in sensitivity to neurohypophyseal hormones have been recognized (Melin, 1970; Knight, 1974a; Allison *et al.*, 1988) that could explain agonist and antagonist effects described above.

Filippi *et al.* (2005) have recently hypothesized, that ET-1-induced contractions in the rabbit epididymis can be abolished by both an oxytocin antagonist and atosiban. However, although large robust ET-1 contractions could be induced in the present study, no inhibition was observed with L-368899 or SR49059, implying that ET-1 does not release endogenous oxytocin or AVP to mediate the contraction. It remains to be determined whether the peptide non-selective oxytocin antagonists have the ability to displace the peptide ET-1 or whether the interaction of oxytocin antagonists on ET-1-induced contractions observed within

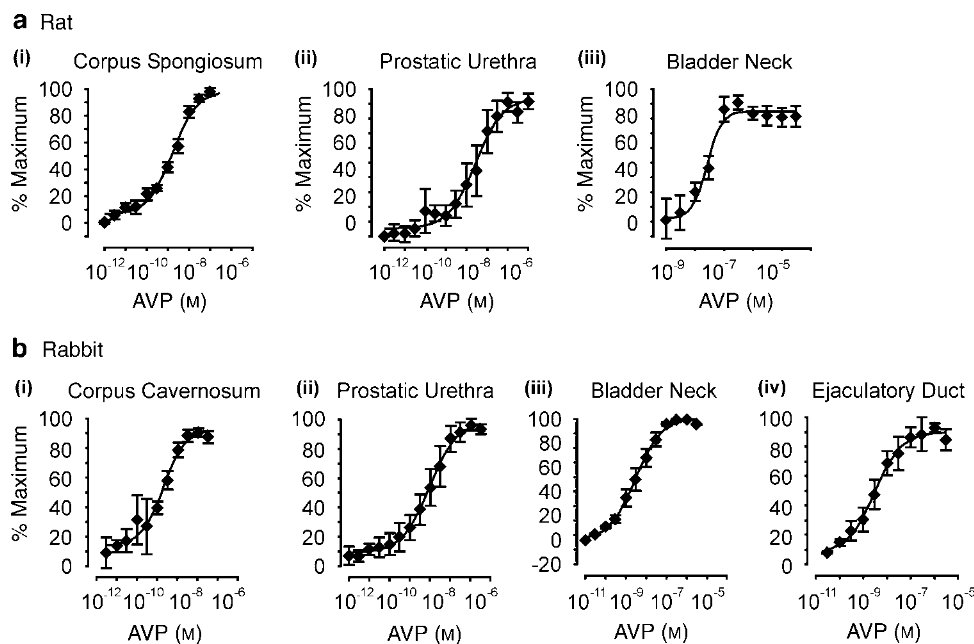

Figure 4 Effect of arginine vasopressin (AVP) on rat and rabbit reproductive tissues *in vitro*. Concentration–response curves showing AVP-induced contractions in (i) corpus spongiosum/cavernosum, (ii) prostatic urethra (iii) bladder neck and (iv) ejaculatory duct dissected from (**a**) rat and (**b**) rabbit.

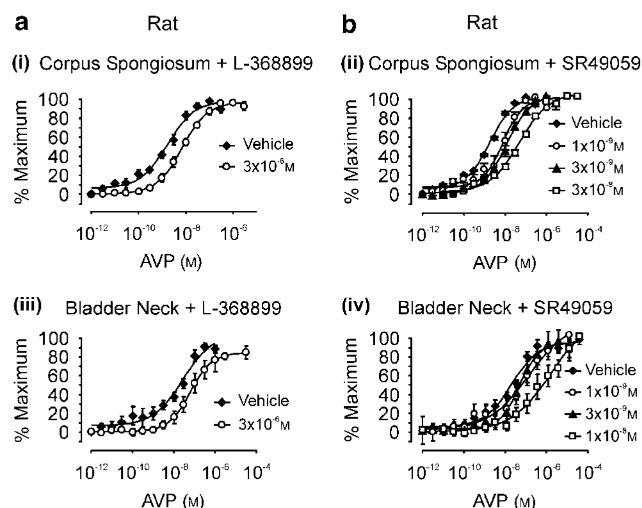

Figure 5 Effect of arginine vasopressin (AVP) in the presence of the oxytocin antagonist L-368899 and the V_{1A} antagonist SR49059 on rat reproductive tissues. AVP concentration–response curves in rat (i) corpus spongiosum and (ii) bladder neck tissues in the absence (vehicle) or presence of (**a**) L-368899 (3×10^{-6} M) and (**b**) varying concentrations of SR49059.

the rabbit epididymis was solely due to rabbits being pretreated with oestrogen to change sex steroid milieu unlike the present study.

Until recently, there is a paucity of native human data, which make it difficult to understand fully how far our findings of oxytocin and AVP effects through V_{1A} receptors in rat and rabbit ejaculatory tissues could be applicable to human tissues. For instance, there have been no studies investigating the contractile effects of oxytocin and AVP in human testes, ampulla or bladder neck. Only AVP has been tested in vas deferens where no tonic contraction was observed but this peptide evoked large phasic contractions and potentiated nerve-evoked contractions. These effects were through a V_1-like receptor, based on antagonism with a high concentration of a peptide antagonist but the subtype was not determined (Andersson *et al*., 1988; Medina *et al*., 1998). However, oxytocin and AVP at 1–10 nM have been reported to induce tonic contractions in human prostate, but the receptor subtype has yet to be investigated (Bodanszky *et al*., 1992). Compelling data with oxytocin-induced contractions in human epididymis and corpus cavernosum have been reported but again receptor validation with selective small molecule non-peptides remains to be carried out (Filippi *et al*., 2002b, 2005; Vignozzi *et al*., 2004). It is important to note that these authors also clearly demonstrated that the oxytocin receptor expression and function they observed was oestrogen dependent. Hence, care should be taken in interpreting data from human ejaculatory and erectile tissues that are generally obtained from patients undergoing surgery for sexual reassignment as they will have undergone an extensive period of treatment with oestrogen supplements. Further studies either in non-hormonally treated human tissue *in vitro* or clinical studies in man with V_{1A} antagonists is essential to provide greater insights into how the physiology and pharmacology already published and reported in the present study will translate to patients who are looking for therapeutic invention for premature ejaculation or erectile dysfunction.

In conclusion, this is the first report validating the fact that V_{IA} receptors mediate the oxytocin contractile effect on selective ejaculatory and erectile tissues in both rat and rabbit, suggesting the use of selective V_{IA} antagonists as a dual peripheral therapeutic approach to treat premature

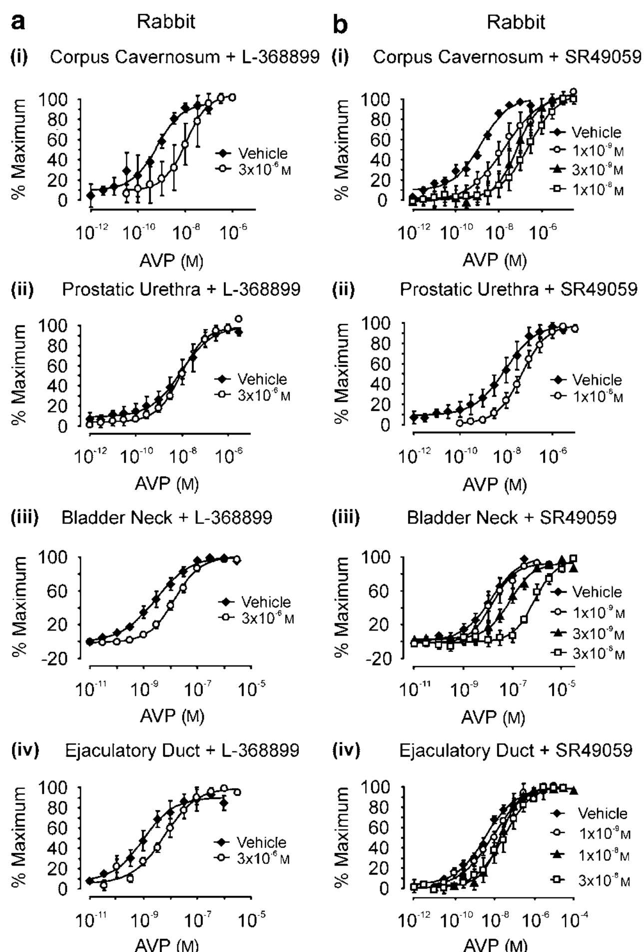

Figure 6 Effect of arginine vasopressin (AVP) in the presence of the oxytocin antagonist L-368899 and the V_{1A} antagonist SR49059 on rabbit reproductive tissues. AVP concentration–response curves in rabbit (i) corpus cavernosum, (ii) prostatic urethra, (iii) bladder neck and (iv) ejaculatory duct tissues in the absence (vehicle) or presence of (**a**) L-368899 (3×10^{-6} M) or (**b**) varying concentrations of SR49059.

Table 4 EC_{50} values for contraction to ET-1 alone and in the presence of L-368899 (100 nM) or SR49059 (10 nM) in rat ejaculatory and erectile tissues

Tissue (rat)	*Control (nM)*	*L-368899 (nM)*	n	*SR49059 (nM)*	n
Corpus spongiosum	16.4 ± 2.5	14 ± 9	6	15.7 ± 2.5	5
Prostatic urethra	36 ± 11.5	55 ± 60	6	812 ± 1148	4
Vas deferens	120 ± 16.3	157 ± 7.2	6	306 ± 93	5
Seminal vesicle	29 ± 6.1	14 ± 5.9	6	42 ± 6.2	5
Testes	6.5 ± 2.9	10.3 ± 3.8	3	470 ± 270	5

Abbreviation: ET-1, endothelin-1.
Contractions to ET-1 shown as mean (± s.e.mean) in rat erectile and ejaculatory tissues in the presence or absence of L-368899 or SR49059 with the number of tissues used.

ejaculation as well as enhance or prolong erections. The species difference in potency to V_{1A} agonists and antagonists in ejaculatory tissues suggests that caution is necessary in predicting potency in humans from animal results.

Conflict of interest

The authors state no conflict of interest.

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