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RESEARCH PAPER

Pharmacological profiling of neuropeptides on rabbit vaginal wall and vaginal artery smooth muscle *in vitro*

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Background and purpose: Hypothalamic neuropeptides centrally modulate sexual arousal. However, the role of neuropeptides in peripheral arousal has been ignored. Vascular and non-vascular smooth muscle relaxation in the vagina is important for female sexual arousal. To date, *in vitro* studies have focused on vaginal strips with no studies on vaginal arteries. The aim of this study was to compare the effects of sexual hypothalamic neuropeptides on rabbit vaginal wall strips and arteries.
Experimental approach: Tissue bath and wire myography techniques were used to measure isometric tension from strips and arteries, respectively.
Key results: Pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) relaxed both preparations, effects that were only antagonized by the VIP/PACAP antagonist VIP6–28 (10 nM) and the PAC_1 antagonist PACAP 6–38 (1 μM). The melanocortin agonist α-melanocortin-stimulating hormone (1 μM), but not bremelanotide (1 μM), also relaxed both preparations. Oxytocin and vasopressin contracted vaginal preparations, which could be antagonized by the V_{1A} antagonist SR 49059. Neuropeptide Y (NPY) and the NPY Y_1 agonist Leu^{31}, Pro^{34} NPY only contracted arteries, which was antagonized by the NPY Y_1 receptor antagonist BIBP 3226. Melanin-concentrating hormone (MCH; 1 μM) contracted arteries.
Conclusion and implications: Hypothalamic neuropeptides can exert contractile and relaxant effects on vaginal strips and arteries. NPY Y_1, V_{1A}, MCH_1 antagonists as well as VIP/PAC_1 agonists may have therapeutic potential in both central and peripheral female sexual arousal. Differences in effect of neuropeptides between preparations raise the question of which preparation is important for female sexual arousal.
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Keywords: female sexual arousal; vagina; arteries; neuropeptides; contraction; relaxation

Abbreviations: αMSH, α-melanocortin-stimulating hormone; AVP, vasopressin; IVA, intra-vaginal artery; MCH, melanin-concentrating hormone; NPY, neuropeptide Y; PACAP, pituitary adenylate cyclase-activating polypeptide; V_{1A}, vasopressin 1A; VIP, vasoactive intestinal peptide

Introduction

Extensive preclinical *in vivo* studies have shown that hypothalamic neuropeptides play an important role in the central control of female sexual behaviour. Neuropeptide Y (NPY; Clark, 1992; Marin-Bivens *et al*., 1998) and vasopressin (AVP; Pedersen and Boccia, 2006) have all been shown to inhibit female receptive copulatory behaviour, also known as lordosis or receptivity, whereas oxytocin (Arletti and Bertolini, 1985; Caldwell *et al*., 1986, 1989; Caldwell, 1992; Benelli *et al*., 1994; Pedersen and Boccia, 2002), pituitary adenylate cyclase-activating polypeptide (PACAP; Apostolakis *et al*., 2004; Apostolakis *et al*., 2005), melanin-concentrating hormone (MCH; Gonzalez *et al*., 1996) and α-melanocortin-stimulating hormone (αMSH; Cragnolini *et al*., 2000; Gonzalez *et al*., 1998; Gonzalez *et al*., 1996; Nocetto *et al*., 2004; Pfaus *et al*., 2004) have all been shown to facilitate female sexual receptivity when injected into the paraventricular nucleus, medial preoptic area or ventromedial nucleus.

Surprisingly to date, although most effort has focused on the role of neuropeptides in centrally mediated sexual arousal, there has been little focus on neuropeptides underlying the peripheral control of female sexual arousal. Vascular and non-vascular smooth muscle relaxation play a critical role during sexual arousal and deficits in these mechanisms during sexual arousal may underlie the pathophysiology of female sexual arousal disorder. During sexual arousal, the vaginal blood vessels relax allowing increased

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blood flow into the vagina, clitoris and external genital organs. The increase in flow leads to increased vaginal lubrication by increased plasma transudation. It is thought that there are increases in genital sensitivity during sexual arousal; however, the underlying mechanisms are poorly understood. The rabbit has generally been used as a preclinical *in vivo* model to investigate vaginal blood flow as a physiological measure of peripheral sexual arousal (Park *et al*., 1997, 2001; Min *et al*., 2001, 2002, 2003; Kim *et al*., 2002, 2003; Angulo *et al*., 2004). Most studies have focused on investigating the role of the nitric oxide–cGMP pathway in controlling vaginal smooth muscle tone, demonstrating the ability of phosphodiesterase type-5 inhibitors to relax vaginal and clitoral muscles and potentially facilitate arousal in women (Sandner *et al*., 2007). To date, only sildenafil has been investigated clinically for effects on female sexual function with disappointing results (Sandner *et al*., 2007). However, there have been limited studies investigating the effect of neuropeptides on vaginal blood flow with most emphasis on vasoactive intestinal peptide (VIP; Levin, 1991). Likewise, there is a paucity of data using non-vascular vaginal smooth muscle strips to study the functional effects of neuropeptides on sexual arousal. Following histochemical identification of VIP, PACAP, peptide histidine methonine, peptide histidine valine and helospectin in nerves of the vagina (Graf *et al*., 1995; Hoyle *et al*., 1996), Ziessen *et al*. (2002) have demonstrated that each of these neuropeptides can directly relax the rabbit vagina when bath applied to pre-contracted vaginal strips. No *in vitro* studies, investigating the effect of neuropeptides directly on vaginal arteries contributing to vaginal blood flow, have been performed.

The aims of the study therefore were to (a) investigate the effect of pro-sexual and anti-sexual hypothalamic neuropeptides in the rabbit vagina *in vitro*, (b) compare potency and efficacy of neuropeptides between vaginal strips and arteries and (c) identify receptor subtypes underlying neuropeptide responses.

Methods

Rabbit vagina preparation

All experiments were carried out in compliance with the UK legislation and subject to local ethical review. Female New Zealand rabbits (~3 kg) were sacrificed by an overdose of pentobarbitone (Pentoject, Animalcare, York, UK) injected into the marginal ear vein. The vagina was removed and placed into Krebs solution (mM): NaCl 119, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11, at 37 °C and gassed with 95% O_2/5% CO_2.

Vaginal strip dissection and mounting

The vagina was dissected into four longitudinal strips from the lower 3 cm of the vagina and then cut in half horizontally to produce four upper and four lower strips. Strips were mounted in 5 mL organ baths containing Krebs solution aerated with 95% O_2/5% CO_2 and allowed to equilibrate under a resting tension of 19.6 mN for 1 h.

Vaginal artery dissection and mounting

Following removal of the vagina, arteries were isolated and dissected into 2 mm rings. The artery entering the vagina is referred to as the 'extra-vaginal artery' and the artery within the lower 2 cm of the vagina is referred to as the 'intra-vaginal artery' (IVA). Using wire myography, isometric recordings were made from vessel rings (i.d. of extra-vaginal artery: 263 ± 15 μM; IVA: 173 ± 3.6 μM) equilibrated for 30 min under a resting tension of 2.94 mN. All experiments were performed in Krebs solution at 37 °C and gassed with 95% CO_2/5% O_2. Following equilibration, arterial rings were pre-contracted three times using phenylephrine (10 μM) followed by a 30 min washout period before neuropeptide application.

Concentration response curves

To investigate the contractile effect of neuropeptides in vaginal strips and arteries, agonist concentration response curves were obtained by cumulatively applying half-log concentration increments. A washout period for 1 h was followed by either vehicle or antagonist incubation for a further 20 min before repeating a reproducible concentration response curve.

For relaxation experiments, vaginal strips and arteries were pre-contracted with phenylephrine (10–20 μM). Neuropeptide agonist concentration response curves were obtained by cumulative half-log additions. A 1 h washout period was followed by either vehicle or antagonist incubations for 20 min before repeating a reproducible concentration response curve.

Data analysis

Data are expressed as mean ± s.e. of the mean (s.e.mean) and *n* indicates the number of vaginal strips/arterial rings. The EC_{50}, slope and pK_B were determined using Labstats (Excel add-in, Pfizer). Experimental data using three antagonist concentrations were analysed to obtain pK_B values using the global nonlinear regression method described by Lew and Angus (1995). Advantages offered by this method over the traditional Schild method have been discussed previously (Lew and Angus, 1995), briefly the method does not require the within-tissue control concentration response curves required for standard Schild regression methods. In experiments, where the antagonist was known to be competitive, a single antagonist concentration was used to antagonize the agonist concentration response curve and the Gaddum equation (http://www.pdg.cnb.uam.es/cursos/Barcelona2002/pages/Farmac/Comput_Lab/Guia_Glaxo/chap2d.html#pkb) was used. Statistical significance was determined by Students *t*-test or ANOVA.

Compounds

Compounds were obtained from the following sources: phenylephrine, AVP, oxytocin, BIBP 3226 and MCH were obtained from Sigma-Aldrich (Gillingham, Dorset, UK). NPY (human, rat), PACAP 6–38, GR231118, NPY (13–36), [cPP_{1-7}, NPY_{19-23}, Ala^{31}, Aib^{32}, Gln^{34}]-h pancreatic polypeptide PYY (3–36), Leu^{31},Pro^{34} NPY were supplied by Bachem

(St Helens, Merseyside, UK) and VIP, VIP6–28, PACAP 1–27, PACAP 1–38 and αMSH from Tocris (Avonmouth, Bristol, UK). SR 49059, L-368899 and bremelanotide were synthesized as reported in the literature (Serradeil-Le Gal *et al*., 1993; Thompson *et al*., 1997; Blood *et al*., 2001).

Results

Neuropeptide-evoked contractions in rabbit vascular and non-vascular smooth muscle

To investigate the effect of neuropeptides in the rabbit vagina, neuropeptides were added to the organ bath. AVP (0.01–100 nM) and oxytocin (0.1 nM–3 μM) elicited concentration-dependent contractions in upper vaginal strips with a maximum contraction of 5.7 ± 0.7 mN ($n = 28$) and 12.4 ± 2.2 mN ($n = 13$), respectively (Figures 1a and b). Neither peptide contracted the lower 1.5 cm vaginal strips. The AVP-induced contraction was 20-fold more potent than oxytocin as summarized in Table 1. As shown in Figure 1, AVP (0.01 nM-100 nM) and oxytocin (0.1 nM–10 μM) also contracted all regions of the rabbit vaginal artery with a maximum contractile response of 6.3 ± 0.5 mN ($n = 53$) and 5.7 ± 0.5 mN ($n = 49$), respectively. As in the vaginal strips, AVP was more potent than oxytocin (see Table 2). Both neuropeptides were more potent in the vaginal arteries compared to strips of vaginal smooth muscle, with AVP being 15-fold and oxytocin 3-fold more potent (Figure 1 aiii and biii, Table 2).

In contrast to AVP and oxytocin, NPY failed to elicit a concentration-dependent contraction in vaginal strips ($n = 6$; $P > 0.05$). However, NPY potently elicited a large concentration-dependent contraction in vaginal arteries (Table 2). The NPY-induced contractions were greater in the IVA (4.3 ± 0.7 mN, $n = 25$) compared to the extra-vaginal artery (0.7 ± 0.2 mN, $n = 9$; $P < 0.05$).

Similar to NPY, the endogenous MCH receptor agonist MCH had no effect on vaginal strips ($n = 8$) but elicited a large contraction in arterial rings at high concentrations (1 μM, $E_{max} = 3.7 \pm 0.6$ mN, $n = 13$).

Neuropeptide-evoked relaxations in rabbit vascular and non-vascular smooth muscle

As previously shown by Ziessen *et al*. (2002), VIP (0.1 nM–3 μM) and PACAP 1–27 (0.1 nM–3 μM) relaxed pre-contracted vaginal strips (Figure 2). VIP and PACAP 1–27 elicited a maximum relaxation of 87 ± 5.3% ($n = 18$) and 95.9 ± 10.5% ($n = 12$), respectively. Both concentration response curves were shallow (Table 3). VIP ($n = 36$) and PACAP 1–27 ($n = 11$) also fully relaxed pre-contracted vaginal arteries (Table 4; Figure 2). Both neuropeptides response had a slope close to unity and were >20-fold more potent than relaxations

Table 1 Neuropeptide-induced contraction in rabbit vaginal strips

Peptide	*EC_{50} (nM)*	*Slope*	*E_{max} (mN)*	n
AVP	9.5 ± 1.8	1.5 ± 0.2	5.7 ± 0.7	28
Oxytocin	206 ± 83.2	0.8 ± 0.1	12.4 ± 2.2	13
NPY	NE	NE	NE	6
MCH	NE	NE	NE	8

Abbreviations: AVP, vasopressin; MCH, melanin-concentrating hormone; NPY, neuropeptide Y; NE, no effect.

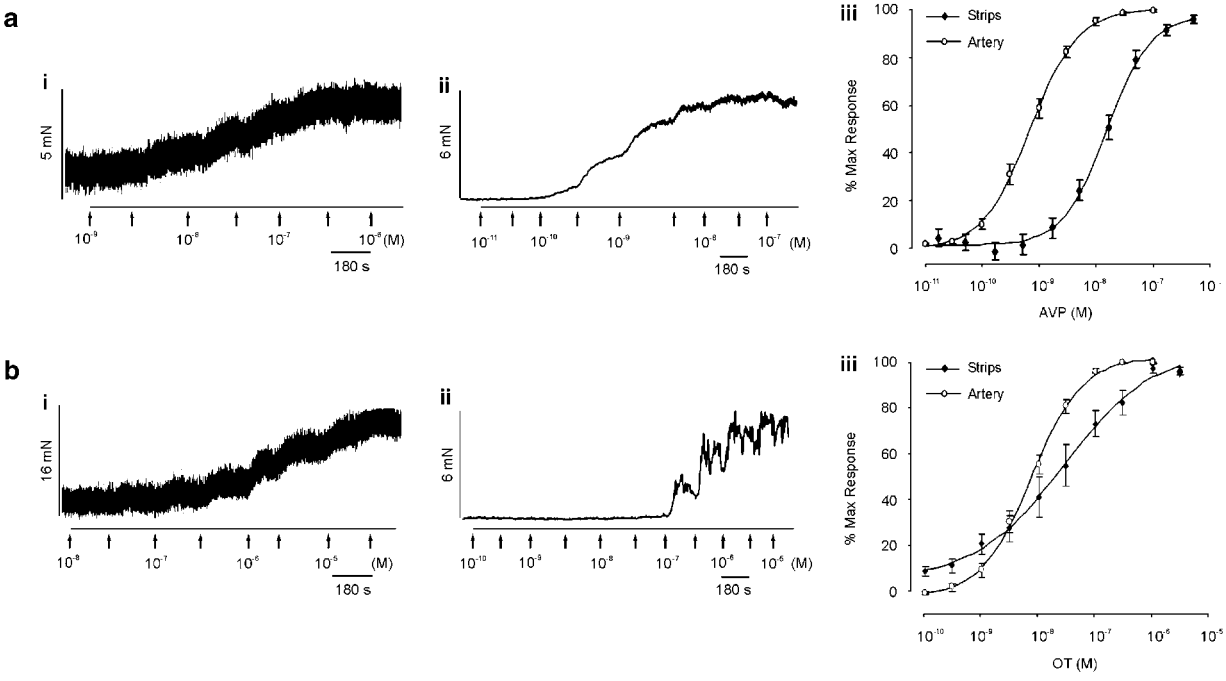

Figure 1 Effect of oxytocin (OT) and vasopressin (AVP) on rabbit vaginal strips and arteries *in vitro*. Panel (**a**) shows an experimental trace of increasing concentrations of AVP on (i) vaginal strips and (ii) vaginal arteries. Panel (**a**) iii is the average concentration response curve to AVP in both tissues. Panel (**b**) is the same as (**a**), but in the presence of oxytocin.

observed in non-vascular tissue strips. PACAP 1–38 failed to relax either non-vascular or vascular smooth muscle ($n=7$; $P>0.05$).

The non-selective melanocortin agonist, αMSH, relaxed both vaginal strips and arteries; an effect that was not concentration-dependent and was only elicited at 1 μM (36 ± 6.7% ($n=12$) in vaginal strips and 43.6 ± 10.2% ($n=12$) in arteries). In contrast, no effect was observed with another non-selective melanocortin agonist, bremelanotide (1 μM, $n=4$–5; $P>0.05$).

Characterization of receptor subtype(s) underlying AVP and oxytocin-induced contractions in non-vascular and vascular smooth muscle

To determine whether oxytocin was acting through oxytocin receptors on vascular and non-vascular smooth muscle, the potent oxytocin antagonist L-368899 was used ($K_i=3.6$ nM; Thompson *et al*., 1997). L-368899 (10–100 nM) failed to shift the oxytocin concentration response curve in low concentrations. Only at high, non-selective, micromolar concentrations were there effects in vaginal arteries (oxytocin, EC_{50} 75 ± 3.9 nM; oxytocin and 1 μM L-368899 EC_{50} 300 ± 43 nM, $n=10$; $P<0.01$; Figure 3a) and in strips (oxytocin EC_{50} 206.4 ± 83.2 nM; oxytocin and 3 μM L-368899 EC_{50} 1.8 ± 0.8 μM, $n=10$; $P<0.05$; Figure 3a). As observed with oxytocin, only 1 μM L-368899 antagonized the AVP concentration response curves in vaginal arteries (AVP EC_{50} 1.2 ± 0.07 nM; AVP and 1 μM L-368899 EC_{50} 6.2 ± 0.5 nM, $n=4$, $P<0.05$), but failed to affect AVP at concentrations up to 3 μM in vaginal strips ($n=4$; Figure 3b).

As L-368899 did not potently antagonize the oxytocin-induced contractions in vaginal tissues, it was possible that

Table 2 Neuropeptide-induced contraction in rabbit vaginal artery

Peptide	*EC_{50} (nM)*	*Slope*	*E_{max} (mN)*	n
AVP	0.6 ± 0.1	1.1 ± 0.1	6.3 ± 0.5	53
Oxytocin	76.9 ± 6.4	1.4 ± 0.1	5.7 ± 0.5	49
NPY	26.9 ± 4	0.9 ± 0.1	4.3 ± 0.7	25
MCH	>1000	—	3.7 ± 0.6	13

Abbreviations: AVP, vasopressin; MCH, melanin-concentrating hormone; NPY, neuropeptide Y.

Table 3 Neuropeptide-induced relaxation in rabbit vaginal strips

Peptide	*EC_{50} (nM)*	*Slope*	*E_{max} (mN)*	n
VIP	80.9 ± 5.4	0.60 ± 0.3	87.0 ± 5.3%	18
PACAP 1–27	116 ± 82.3	0.4 ± 0.2	95.9 ± 10.5%	12
αMSH	>1000	—	36 ± 6.7%	12

Abbreviations: αMSH, α-melanocortin-stimulating hormone; PACAP, pituitary adenylate cyclase-activating polypeptide; VIP, vasoactive intestinal peptide.

Table 4 Neuropeptide-induced relaxation in rabbit vaginal arteries

Peptide	*EC_{50} (nM)*	*Slope*	*E_{max} (mN)*	n
VIP	4.8 ± 0.2	1.1 ± 0.1	97.9 ± 5.5%	36
PACAP 1-27	3.7 ± 0.3	1 ± 0.1	100.4 ± 0.7%	11
αMSH	>1000	—	43.6 ± 10.2%	12

Abbreviations: αMSH, α-melanocortin-stimulating hormone; PACAP, pituitary adenylate cyclase-activating polypeptide; VIP, vasoactive intestinal peptide.

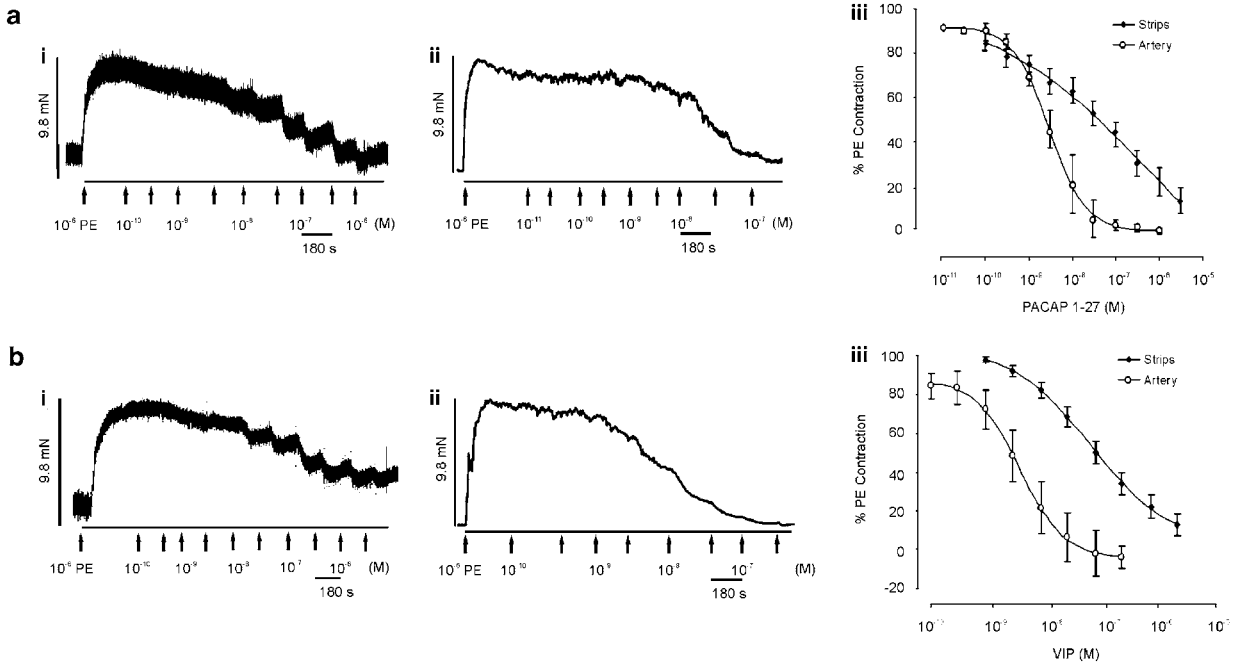

Figure 2 Effect of pituitary adenylate cyclase-activating polypeptide (PACAP 1–27) and vasoactive intestinal peptide (VIP) on rabbit vaginal strips and arteries *in vitro*. Panel (**a**) shows an experimental trace of increasing concentrations of PACAP 1–27 on (i) vaginal strips and (ii) vaginal arteries, pre-contracted with phenylephrine (PE). Panel (**a**) iii shows the average concentration response curve to PACAP 1–27 in both tissues. Panel (**b**) is the same as (**a**), but in the presence of VIP.

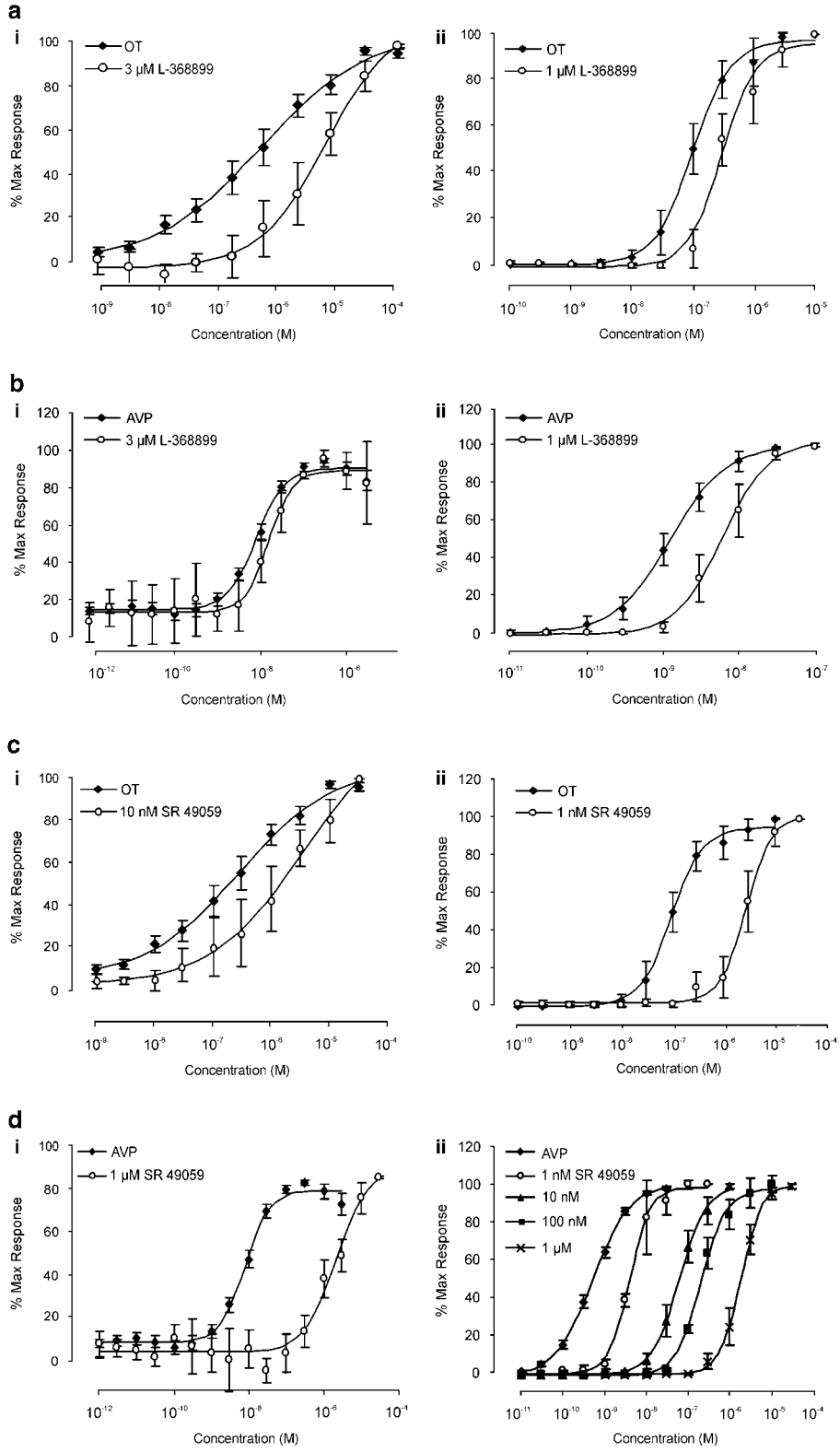

Figure 3 Effect of OT and AVP in the presence of L-368899 and SR 49059 in vagina strips and arteries. The left hand column shows the effect of both OT and AVP in the presence of either the OT antagonist L-368899 (a and b) or the V1A antagonist SR 49059 (c and d) on vagina strips. Graphs in the right hand column are the same as the left hand column but on vagina arteries.

oxytocin was acting through the vasopressin 1A (V_{1A}) receptor. The selective non-peptide V_{1A} antagonist SR 49059 (10 nM; $K_i = 1.6$; Serradeil-Le Gal *et al.*, 1993) caused a fivefold shift of the oxytocin concentration response curve in vaginal strips ($pK_B = 8.7$, $n = 5$) with an apparent $pK_B = 10.2$ ($n = 6$) in vaginal arteries as shown in Figure 3c.

Similarly, AVP-induced contractions were potently antagonized by SR 49059 in vaginal strips (apparent $pK_B = 8.15$, $n = 5$; Figure 3d) and vaginal arteries ($pK_B = 9.63$, $n = 8$; Figure 3d).

Identification of receptor subtype underlying NPY-induced contraction in the rabbit vaginal artery

A range of NPY selective agonists were tested on the rabbit vaginal artery including NPY (13–36) (Y_2 agonist), GR231118 (Y_4 agonist), [cPP_{1-7}, NPY_{19-23}, Ala^{31}, Aib^{32}, Gln^{34}]-h pancreatic polypeptide (Y_5 agonist) and the non-selective agonist PYY (3–36). All failed to elicit a contraction (1 μM; $n = 3$–4; $P > 0.05$). However, the Y_1 agonist, Leu^{31}, Pro^{34} NPY induced a concentration-dependent contraction that was of similar amplitude to that of NPY (EC_{50} 38.9 ± 14.1 nM, slope 1.1 ± 0.2, E_{max} 4.5 ± 1.6 mN, $n = 11$; Figure 4a). To fully investigate the presence of NPY Y_1 receptors, the selective peptide NPY Y_1 antagonist BIBP 3226 was tested against NPY in the IVA only (rabbit $pK_B = 6.98$; Doods *et al*., 1995). BIBP 3226 competitively inhibited the NPY contractile response (apparent $pK_B = 7$, slope 0.7, $n = 3$–9; Figure 4b).

Characterization of receptor subtype(s) underlying VIP and PACAP-induced relaxations in non-vascular and vascular smooth muscle

The selective peptide PAC_1 receptor antagonist PACAP 6–38 (1 μM) and VIP/PACAP antagonist VIP6–28 (10 nM) failed to block either PACAP 1–27 or VIP-induced relaxations in rabbit vaginal strips ($n = 6$; $P > 0.05$) and extra-vaginal artery ($n = 8$; $P > 0.05$). In contrast, in the IVA both VIP6–28 (10 nM) and PACAP 6–38 (1 μM) caused about a four-fold rightward shift in the VIP EC_{50} ($n = 5$–8; Figure 5; $P > 0.05$).

Discussion and conclusions

Novel findings in this study are (a) AVP and oxytocin contract both rabbit vaginal strips and arteries through V_{1A} receptors, (b) AVP and oxytocin have a greater affinity for receptors present in vaginal artery than non-vascular smooth muscle, (c) V_{1A} antagonists are equipotent in vascular and non-vascular smooth muscle, (d) NPY does not contract vaginal strips but potently contracts vaginal arteries through NPY Y_1 receptors, (e) MCH contracts vaginal arteries and not

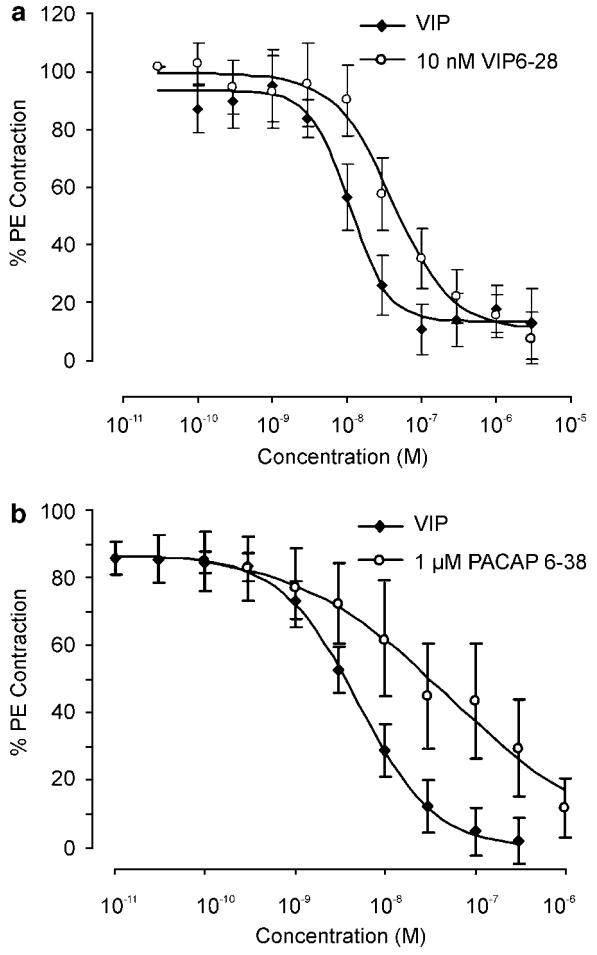

Figure 5 Inhibition of vasoactive intestinal peptide (VIP)-induced relaxations with VIP6–28 and pituitary adenylate cyclase-activating polypeptide (PACAP 6–38) in vaginal arteries. Panel (**a**) shows the effect of the VIP/PACAP antagonist VIP6–28 (10 nM) on the concentration response curve to VIP in vaginal arteries pre-contracted with phenylephrine (PE). Panel (**b**) is the same as (**a**), but in the presence of the PAC_1 antagonist PACAP 6–38 (1 μM).

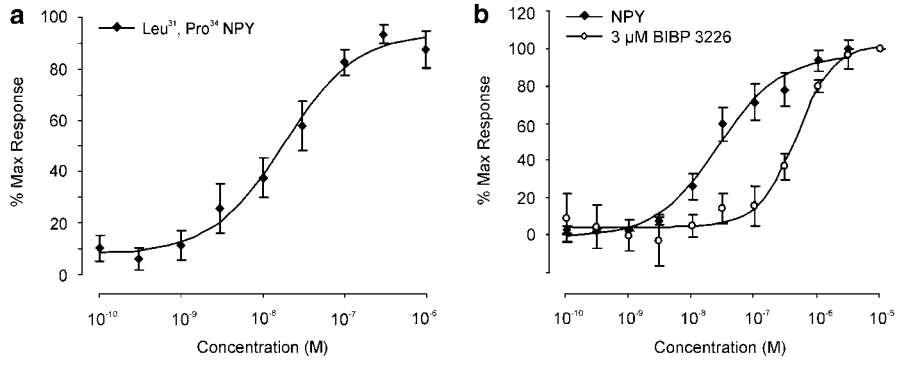

Figure 4 Characterization of neuropeptide Y (NPY) Y_1 receptors in vaginal arteries. Panel (**a**) is the concentration response curve to the NPY Y_1 agonist Leu^{31}, Pro^{34} NPY, in vaginal arteries. Panel (**b**) shows the effect of the peptide NPY Y_1 antagonist BIBP 3226 (3 μM) on the concentration response curve to NPY.

non-vascular vaginal wall, (f) PACAP 1–27 and VIP relax pre-contracted vaginal strips and arteries through different receptor subtypes, (g) PACAP and VIP have a greater affinity for arteries than vaginal strips and (h) αMSH, but not bremelanotide, relaxes both vascular and non-vascular vaginal smooth muscle.

There is much published work showing that oxytocin through the oxytocin receptor has the ability to increase female receptivity (Witt and Insel (1991); Caldwell *et al*., 1994; Pedersen and Boccia, 2002). In addition, it has been reported that plasma oxytocin levels fluctuate throughout the menstrual cycle in women and significantly relates to genital lubrication; implying its role in peripheral activation of sexual function (Salonia *et al*., 2005). However, in contrast, in this study, oxytocin contracted both vaginal strips and arteries implying oxytocin peripherally would dampen blood flow. The lack of potency of oxytocin and failure of a selective oxytocin antagonist to antagonize oxytocin implies oxytocin-induced contractions are not through the oxytocin receptor. These findings are supported by Maggi *et al*. (1988), who by using oestrogenized rabbit vaginal strips showed that oxytocin only contracted the tissue at micromolar concentrations. In addition, binding experiments revealed oxytocin receptors are not present in the rabbit vagina. In this study, the potent contractile effect of AVP and potent antagonism of both oxytocin and AVP by SR 49059 implies the presence of a V_{1A} receptor, which is anti-arousal. Recently, it has been shown that V_{1A} antagonism in the hypothalamus is pro-sexual (Pedersen and Boccia, 2006). Together, these data support the hypothesis that V_{1A} antagonists have the potential to enhance sexual arousal both centrally and peripherally.

It is also well known that NPY released from the forebrain into the hypothalamus is anti-sexual. However, the receptor subtype underlying the central actions of NPY remains to be elucidated. Peripherally, NPY has been shown to modulate uterine blood flow (Tenmoku *et al*., 1988) and immunohistochemical studies have also shown that NPY is found in abundance in the human vagina (Jorgensen *et al*., 1989; Hoyle *et al*., 1996). However, in this study, NPY failed to contract vaginal strips. Interestingly, NPY did contract vaginal arteries through the NPY Y_1 receptor. NPY-induced contractions were greater in the IVA, which may be due to an increase in the density of NPY Y_1 receptors with decreasing size of vessel. These data suggest NPY Y_1 antagonists would have the ability to enhance vaginal blood flow.

Further distinction between vascular and non-vascular smooth muscle pharmacology was observed with MCH, which only contracted vaginal arteries. The receptor subtype underlying the response to MCH is presumably the MCH_1 receptor, as rabbit and other non-primates do not express functional MCH_2 receptors (Tan *et al*., 2002). Further evaluation awaits the availability of a commercial selective MCH_1 antagonist. The present data contrast with the role of central MCH, which is believed to enhance female sexual behaviour (Gonzalez *et al*., 1996, 1998).

As previously reported application of exogenous VIP and PACAP 1–27 relaxed the vaginal wall (Ziessen *et al*., 2002). In contrast, PACAP 1–38 failed to have an effect. VIP and PACAP 1–27 were also significantly more potent in the IVA compared to the vaginal wall, which may be attributed to increased receptor density, different coupling efficiency or a difference in receptor subtypes. The blood vessel data support a clinical case study showing that VIP increases vaginal blood flow and lubrication in healthy women (Levin, 1991). Lack of blockade of both agonists with classical peptide PAC_1 and VIP/PACAP antagonists within the vaginal strips, although these antagonists were efficacious in the IVA, may imply different receptor subtypes. Emerging research in this novel class B receptor family have shown that multiple splice variants exist, which also have differential signal-transduction pathways (Spengler *et al*., 1993; Dickson *et al*., 2006). In addition to PAC_1/VIP receptor agonism enhancing peripheral sexual arousal, it has recently been shown that PACAP acting through PAC_1 receptors within the hypothalamus can increase sexual arousal (Apostolakis *et al*., 2005).

Activation of central melanocortin receptors has been proposed to restore female sexual arousal, both preclinically in rats and also in ongoing clinical trials in women with female sexual arousal disorder. The non-selective peptide melanocortin agonist bremelanotide increased solicitative proceptive copulatory behaviours (hops and darts) in rats and increased desire, arousal and satisfaction in proof-of – concept studies (http://www.palatin.com/pdfs/bremelanotide.pdf; Pfaus *et al*., 2004). This study is the first report of the ability of αMSH to directly relax vascular and non-vascular smooth muscle; however, this relaxation effect was not reproduced by bremelanotide. This conflicting data may be due to species differences in potency and efficacy of melanocortin agents. Alternatively, a non-selective novel mechanism of action for αMSH may have been revealed, which warrants further investigation.

In conclusion, increased understanding of the physiology and pharmacology of female sexual arousal is key to helping us understand the underlying pathophysiology of female sexual dysfunction. At present, female sexual arousal is subdivided into subjective (mental) and physical (genital) arousal. Currently, the pathophysiology of female sexual dysfunction is unclear and may be a result of a genital deficit, as in male erectile dysfunction, or a psychogenic/centrally mediated deficit such as anxiety and depression.

Therapeutic options would be to (a) increase genital arousal that is, increased genital blood flow/lubrication; (b) increase central subjective arousal that is, subjective arousal, increased desire and improved satisfaction or (c) increase both genital and central subjective arousal. When one combines the data from this study with behavioural data, it is possible to suggest that NPY Y_1 and MCH_1 antagonists may be helpful for restoring genital arousal. V_{1A} antagonists as well as MCH and oxytocin agonists may be helpful in restoring subjective arousal, whereas melanocortin, VIP/PACAP agonists, V_{1A} and NPY_1 antagonists may be useful for the restoration of both genital and subjective arousal. Owing to our poor understanding of female sexual dysfunction, it maybe necessary to test a number of these hypotheses in women to find an effective therapy for the treatment of female sexual dysfunction and restoration of normal sexual function.

Conflict of interest

The authors state no conflict of interest.

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