Oxytocin and V1 vasopressin receptors in rabbit endometrium during pregnancy

M. Maggi, A. Peri, S. Giannini, G. Fantoni, V. Guardabasso* and M. Serio

*Department of Clinical Physiopathology, Endocrinology Unit, University of Florence, Viale Morgagni 85, 50134 Florence, Italy; and *Istituto di Ricerche Farmacologiche Mario Negri, Consorzio Mario Negri Sud, 66030 S. Maria Imbaro, Italy*

Summary. Neurohypophyseal hormone receptors were identified and characterized in rabbit endometrium and decidua by radioligand binding methods. The results strongly support the presence of a heterogeneity of sites in the decidua of parturient rabbits. The oxytocin site (R_1) binds oxytocin and oxytocin analogues ([Thr⁴, Gly⁷]oxytocin and OTA) with high affinity, whereas the AVP site (R_2) was selective for the V1 AVP analogues, [Phe², Orn⁸]VT and d(CH₂)₅TyrMeAVP. The concentration of oxytocin receptors was low (50–100 fmol/mg protein) at oestrus (Day 0) and on Day 29 of pregnancy, but increased significantly (about 8-fold, $P < 0.05$) during parturition. Conversely, V1 AVP receptors were more concentrated than the oxytocin sites at the end of pregnancy (150 fmol/mg protein) but did not change during parturition. These results indicate that neurohypophyseal hormones have specific receptors not only in the myometrium but also in the uterine mucosa and we suggest that these receptors may participate in the regulation of uterine activity during pregnancy.

Keywords: oxytocin; vasopressin; endothelin; endometrium; rabbit; pregnancy; parturition

Introduction

Oxytocin is a neurohypophyseal hormone that plays important roles in reproduction and lactation. Oxytocin acts on specific receptors present on myometrial and myoepithelial cells (Soloff *et al.*, 1977) and stimulates contraction. The sensitivity of target tissues such as uterus or mammary gland to oxytocin changes during pregnancy or lactation. This has been related to specific modifications of the number of oxytocin receptors on the smooth muscle cells (Soloff *et al.*, 1979). Caldeyro-Barcia & Posiero (1959) found an 8-fold increase in responsiveness of human uterus to oxytocin between the 20th and 39th week of pregnancy. A concomitant increase in myometrial oxytocin receptors, that parallels the increase in uterine spontaneous activity, was then demonstrated in pregnant women (Fuchs *et al.*, 1982; Maggi *et al.*, 1990) and rodents (Soloff *et al.*, 1977; Fuchs *et al.*, 1983; Riemer *et al.*, 1986; Maggi *et al.*, 1988b; Chan *et al.*, 1990). These findings strongly indicate that oxytocin is involved in the initiation and maintenance of parturition. Vasopressin also induces uterine contraction, acting through V1 vasopressin receptors present in the myometrium of rabbits (Maggi *et al.*, 1988b), rats (Chan *et al.*, 1990) and humans (Maggi *et al.*, 1990). However, in contrast to the oxytocin receptors, the concentration of V1 vasopressin receptors is only slightly affected by physiological events such as pregnancy and delivery. Therefore, the physiological significance of these sites in myometrium is still debated.

Oxytocin receptors have been also identified in the endometrium of several animal species (Roberts *et al.*, 1976; Sheldrick & Flint, 1985; Meyer *et al.*, 1988; Soloff & Fields, 1989), including humans (Fuchs *et al.*, 1982, 1984), and have been characterized in the endometrium of the ewe (Ayad & Wathes, 1989). Compelling evidence indicates that these sites are involved in the control of endometrial prostaglandin (PG) production (Roberts *et al.*, 1976; Fuchs, 1987), via the

hydrolysis of phosphoinositides (Flint *et al.*, 1986). Conversely, no specific receptors for arginine vasopressin (AVP) have been demonstrated in the endometrium, although AVP as well as oxytocin stimulated inositol phosphate production in human decidua cells (Schrey *et al.*, 1986). We have recently reported that endothelin-1 is produced by endometrial cells of rabbits in primary culture and that this production is under control of neurohypophysial hormones (Orlando *et al.*, 1990). Endothelin-1 is a potent vasoactive peptide (Yanagisawa *et al.*, 1988) that also increases uterine activity, acting through specific receptors present in rabbit myometrium (Maggi *et al.*, 1991).

The aim of this study was to investigate the presence of neurohypophysial hormone receptors in rabbit endometrium and decidua. These tissues were studied before (Day 0) and during pregnancy (Day 29) as well as during spontaneous delivery, in view of the primary role of neurohypophysial hormones at this time.

Materials and Methods

Animals. Sexually mature New Zealand White rabbits, weighing 3.5–4.5 kg, at oestrus (purple and oedematous vulva) were obtained from a commercial breeder (Malenotti, Pratolino, Florence, Italy): 7 rabbits were mated with fertile bucks and killed after Day 29 of pregnancy (N = 4) or during parturition (N = 3) and 3 rabbits at oestrus were killed before mating (Day 0).

Chemicals. [^3H]AVP (sp. act. 70 Ci/mmol) and [^3H]oxytocin (sp. act. 36 Ci/mmol) were purchased from New England Nuclear (Boston, MA, USA). The tritiated ligands were placed in aliquots in plastic tubes, sealed under nitrogen and frozen at -80°C . AVP, oxytocin and [1-(β -mercapto- β , β -cyclopentamethylenepropionic acid)-2-(O-methyl)Tyr, Arg 8]vasopressin, (d(CH $_2$) $_5$ TyrMeAVP) (VI AVP antagonist; Kruszynski *et al.*, 1980) were obtained from Sigma (St Louis, MO, USA). [Thr 4 , Gly 7]oxytocin (oxytocin agonist; Lowbridge *et al.*, 1977) [Phe 2 , Ile 3 , Orn 8]vasopressin ([Phe 2 , Orn 8]vasotocin) (VI AVP agonist; Huguenin, 1966) and [1-(β -mercapto- β , β -cyclopentamethylenepropionic acid, 2-O-Me-Tyr 3 , Orn 9 , Tyr-amide)]vasotocin (OTA) (oxytocin antagonist; Elands *et al.*, 1988) were obtained from Peninsula Laboratories Inc. (San Carlos, CA, USA).

Membrane preparations. Uterus and oviducts were promptly removed, dissected free of fat and adherent tissue, and plunged into ice-cold 0.9% (w/v) NaCl. Uterine horns were separated from oviduct and cervico-vaginal tract and opened longitudinally. The endometrium or decidua parietalis were separated from the underlining myometrium by scraping with the handle of the scalpel. Membranes were prepared as described previously (Maggi *et al.*, 1986). The uterine mucosa was suspended in Buffer 1 (10 mM-Tris-HCl, pH 7.4, containing 1.5 mM-EDTA, 0.5 mM-dithiothreitol, 1 mM-benzamidine, 0.01% bacitracin and 0.002% soybean inhibitor trypsin), homogenized with a glass-Teflon homogenizer (Thomas Scientific, Swedesboro, NJ, USA: 3 strokes at 1200 r.p.m.) and filtered through cheesecloth. The homogenate was centrifuged at 1000 g for 10 min at 4°C . The supernatant containing the crude membrane fraction was then ultracentrifuged at 160 000 g for 30 min at 4°C .

The resulting pellet was dispersed in Buffer 2 (50 mM-Tris-maleate, pH 7.6, 10 mM-MgSO $_4$, 1 mM-benzamidine, 0.01% bacitracin and 0.002% soybean trypsin inhibitor) and centrifuged again at 160 000 g for 30 min at 4°C . The final pellet was then resuspended in Buffer 2 and divided into small aliquants. The membrane preparation was frozen in solid CO $_2$ and stored at -80°C until assayed. Protein concentration was estimated using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Munchen, West Germany) according to Bradford (1976).

Binding studies. Binding studies were conducted as previously described (Maggi *et al.*, 1988b). Membrane samples (0.3 mg/ml) were incubated with ligands in Buffer 2 in the presence of 0.1% BSA at 22°C for 2 h for tubes containing [^3H]AVP and for 5 h for tubes containing [^3H]oxytocin.

[^3H]AVP and [^3H]oxytocin were present at 1 nM in tubes containing increasing concentrations (0.1–10 000 nM) of unlabelled peptides and at 0.1–1 nM in tubes without unlabelled ligands. The reaction was terminated by rapid filtration through Whatman GF/B filters (Clifton, NJ, USA), presoaked for at least 3 h in ice-cold 50 mM-Tris, pH 7.4, in 0.1% BSA, to minimize filter absorption. A semiautomatic cell-harvesting system (Brandel M-48R, Gaithersburg, MD, USA) was used. Filters were washed twice with 3 ml ice-cold 50 mM-Tris, pH 7.4. Radioactivity retained by filters was measured in a liquid scintillation counter, after overnight incubation in 10 ml scintillation fluid (Insta-gel, Packard Instruments Company Inc, Downers Grove, IL, USA). Self- and cross-displacement curves among [^3H]oxytocin, [^3H]AVP, and several analogues with different specificities for neurohypophysial hormone receptors were prepared, using decidual membranes from parturient rabbits. In additional studies receptor populations were investigated using a multiligand experimental design, in which multiple analogues (labelled ligand, unlabelled ligand, and blocking ligand) were present simultaneously in each assay tube (Maggi *et al.*, 1988a, b).

Analysis of results. Families of self- and cross-displacement curves were analysed simultaneously using the program LIGAND (Munson & Rodbard, 1980). Models of increasing complexity were evaluated (Maggi *et al.*, 1988b). Selection among models was based on the root mean square error of each fit using an F-test based on the "extra-sum-of-square principle" and on tests of randomness of the residuals around the fitted curves (Munson & Rodbard, 1980). Statistical comparisons were performed using one-way analysis of variance (ANOVA) and Tukey's test with the SAS statistical package (SAS Institute Inc., Cary, NC, USA).

Results

Mathematical modelling of the binding data strongly indicated the presence of a heterogeneity of sites in the decidua of parturient rabbits. The introduction of a second independent class of sites significantly improved the goodness of fit ($P < 0.001$). Figure 1 shows a typical family of self- and cross-displacement curves, while binding capacity and affinity constants are given in Table 1. Figure 2 provides a graphical summary of the relative selectivity of the different ligands for the two

Fig. 1. Families of self- and cross-displacement curves between oxytocin (Δ), AVP ($\bullet$), $[\text{Thr}^4, \text{Gly}^7]\text{oxytocin}$ ($\blacksquare$), OTA ($\blacktriangle$), $\text{d}(\text{CH}_2)_5\text{TyrMeAVP}$ ($\circ$) and $[\text{Phe}^2, \text{Orn}^8]\text{vasotocin}$ ($\square$) obtained by the program LIGAND-PC (SCAGRF). In homologous competition curves, the concentrations of the tracers were progressively reduced to improve the characterization of the high affinity region of the binding curves. In the heterologous displacement curves, fixed (1 mM) concentrations of tracers were displaced by increasing concentrations of unlabelled ligands.

Table 1. Concentration of receptors and affinity (K_d) for oxytocin, AVP, OTA, $d(CH_2)_5TyrMeAVP$, $[Thr^4, Gly^7]$ oxytocin and (Phe^2, Orn^8) vasotocin in decidua of parturient rabbits

	Binding site	
	R_1	R_2
Binding capacity (fmol/mg protein)	553 ± 17	61 ± 6.4
Ligand (K_d , nM)		
Oxytocin	0.54 ± 0.03	30.75 ± 8
AVP	2.60 ± 0.21	0.14 ± 0.02
OTA	0.11 ± 0.01	21.04 ± 7.6
$d(CH_2)_5TyrMeAVP$	20.25 ± 2.22	0.07 ± 0.05
$[Thr^4, Gly^7]$ oxytocin	0.34 ± 0.04	1043 ± 438
$[Phe^2, Orn^8]$ vasotocin	5.48 ± 0.6	0.12 ± 0.07

Binding parameters and standard errors were obtained from the simultaneous computer modelling of two families of self- and cross-displacement curves, using the program LIGAND (Munson & Rodbard, 1980).

Fig. 2. Graphical representation of the affinity of oxytocin, AVP and several analogues for the oxytocin site (R_1) and the V1 AVP site (R_2) in the endometrium of parturient rabbits. The K_d values were obtained through the analysis of 16 curves from 2 membrane preparations in 2 competition experiments. Peptides in the region above the shaded area are selective for R_2 (AVP site); peptides below the shaded area are selective for R_1 (oxytocin site).

sites. AVP bound with high affinity to both sites (R_1 and R_2), showing about 10-fold selectivity for R_2 . The V1 agonist $[Phe^2, Orn^8]$ vasotocin and the V1 antagonist $d(CH_2)_5TyrMeAVP$ were the most selective ligands for R_2 . Oxytocin preferentially bound, with about 100-fold selectivity, to the

Table 2. Concentration of receptors and affinity for oxytocin (R_1) and V1 AVP (R_2) in rabbit endometrium

	Endometrium/decidua			
	Oxytocin site		AVP site	
	Binding capacity (fmol/mg protein)	K_d (nM)	Binding capacity (fmol/mg protein)	K_d (nM)
Day 0	90 $\pm$ 75 (3)	0.33 $\pm$ 0.1	18 $\pm$ 1 (3)	0.41 $\pm$ 0.38
Day 29	66 $\pm$ 7 (4)	0.24 $\pm$ 0.07	145** $\pm$ 12 (4)	0.13 $\pm$ 0.04
Parturition	544* $\pm$ 57 (3)	0.29 $\pm$ 0.16	102** $\pm$ 16 (3)	0.19 $\pm$ 0.1

Values (mean $\pm$ s.e.m., no. of observations in parentheses) were derived from the analysis of multiligand experiments in rabbits: at oestrous (Day 0), at the end of pregnancy (Day 29) and during spontaneous parturition. * P < 0.05 compared with Day 0 or Day 29. ** P < 0.05 compared with Day 0, using one-way ANOVA and Tukey's test.

R_1 receptor. The oxytocin agonist [Thr⁴, Gly⁷]oxytocin and the oxytocin antagonist OTA showed high selectivity for R_1 . We therefore conclude that two distinct classes of sites are present in decidual membranes: R_1 that corresponds to the oxytocin site, and R_2 that corresponds to the V1 AVP site.

In attempting to establish the binding capacity of these receptors at different time of pregnancy, we performed 'blocking experiments' (Maggi *et al.*, 1988a, b) in endometrial membranes from non-pregnant and pregnant rabbits. Oxytocin receptors (R_1) were studied using homologous competition curves for oxytocin in the presence of 10 nM-d(CH₂)₅TyrMeAVP. V1 AVP receptors (R_2) were studied using homologous displacement curves for AVP in the presence of 50 nM-[Thr⁴, Gly⁷]oxytocin. This was done to mask respectively the V1 AVP and the oxytocin sites. The results obtained using this experimental design in decidua of parturient rabbits (Table 2) were absolutely consistent with those derived from self- and cross-displacement studies (Table 1). A similar validation of this experimental design was previously reported (Maggi *et al.*, 1988b).

Table 2 illustrates the number of oxytocin and V1 AVP receptors in uterine mucosa at oestrus (Day 0), at the end of pregnancy (Day 29) and at the time of parturition. The binding capacity for oxytocin was approximately the same at oestrus and on Day 29 of pregnancy, but at parturition a dramatic surge (about 8-fold, P < 0.05) in decidual concentration of oxytocin receptors was observed. The capacity of V1 AVP receptors, low at oestrus, increased 8-fold on Day 29 of pregnancy (P < 0.05), but there was no additional increase of V1 AVP sites at the time of parturition.

Discussion

We have identified and characterized the presence of two distinct populations of neurohypophysial hormone receptors in rabbit decidua. The oxytocin site (R_1) binds oxytocin, the selective oxytocin agonist [Thr⁴, Gly⁷]oxytocin and the antagonist OTA. Conversely, the AVP site (R_2) shows selectivity for the V1 AVP agonist [Phe², Orn⁸]vasotocin and the V1 AVP antagonist d(CH₂)₅TyrMeAVP. AVP binds with high affinity to both sites. These results are in perfect agreement with the ligand specificity of oxytocin and V1 vasopressin receptors present in the myometrium of rabbits (Maggi *et al.*, 1988b). Furthermore, our study suggests that oxytocin and V1 vasopressin receptors serve distinct physiological functions. We found a sharp increase in the number of oxytocin receptors in the decidua of parturient rabbits, while V1 AVP sites were unchanged. These findings are in agreement with previous studies on oxytocin and AVP receptors in the myometrium of rodents (Maggi *et al.*, 1988b; Chan *et al.*, 1990) and humans (Maggi *et al.*, 1990). In humans, a parallel rise in myometrial and decidual oxytocin receptors throughout pregnancy was reported

(Fuchs *et al.*, 1982, 1984). These results strongly suggest that the concentration of oxytocin receptors in the uterus plays a critical role during parturition. In rabbits sex steroids may represent one of the physiological signals for the surge in uterine oxytocin receptors at the end of pregnancy: (1) oxytocin receptors are very sensitive to the sex steroid milieu (Soloff, 1975; Soloff *et al.*, 1983); (2) pharmacological treatments of immature rabbits with increasing concentrations of oestradiol-17 β stimulated a dose-dependent increase in myometrial oxytocin sites, while progesterone administration had the opposite effect (Maggi *et al.*, 1988b); (3) an abrupt decline in the progesterone/oestrogen ratio is present at the end of pregnancy (Challis *et al.*, 1973).

The function of oxytocin and VI AVP receptors in the endometrium and decidua is still unclear. Previous studies indicate that oxytocin modulates the endometrial production of prostaglandin (PG), while AVP is ineffective (see for review in Fuchs, 1987). It has been assumed that PGs are essential for the process of parturition, acting both on uterine contraction and cervical dilatation. Therefore, a dual role for oxytocin in parturition was proposed: eliciting myometrial contractions and releasing decidua PGs (Fuchs & Fuchs, 1984). We recently proposed a new mechanism of action of oxytocin on endometrial cells (Orlando *et al.*, 1990). We demonstrated that both oxytocin and VI AVP receptors are involved in the regulation of endothelin secretion from rabbit endometrial cells in primary culture (Orlando *et al.*, 1990). Indeed, the addition of [Thr⁴, Gly⁷]oxytocin and [Phe², Orn⁸]vasotocin elicited an 8- and 2-fold increase, respectively, in endothelin-1 levels. This effect was completely counteracted by pretreatment with the antagonists OTA and d(CH₂)₅-TyrMeAVP. The relative selectivity of these analogues for the neurohypophysial hormone receptors in rabbit endometrium has been clearly shown in this study. Endothelin-1 is a potent uterotonic peptide, which stimulates uterine contractility (Kozuka *et al.*, 1989; Maggi *et al.*, 1991), acting through specific receptors present in myometrium (Maggi *et al.*, 1991). An additional paracrine mediator of oxytocin activity is therefore proposed, i.e. endothelin-1 (Orlando *et al.*, 1990; Maggi *et al.*, 1991). However, the real contribution of this peptide in the initiation of parturition has yet to be assessed.

In conclusion, we have demonstrated that at the time of spontaneous parturition oxytocin, but not AVP receptors increase in the uterine mucosa as well as in the muscular layer. The activation of these receptors by endogenous (maternal or fetal) oxytocin may promote delivery of the fetus directly (contraction of myometrium) or through the stimulation of endometrial prostaglandins or endothelin secretion.

The work was financially supported by a grant from the University of Florence and by a grant from the Consiglio Nazionale delle Ricerche (CNR). The work of M.M. was supported by a special grant from FIDIA Pharmaceuticals, Abano Terme, Italy.

References

- AYAD, V.J. & WATHES D.C. (1989) Characterization of endometrial and myometrial oxytocin receptors in the non-pregnant ewe. *J. Endocr.* **123**, 11–18.
- BRADFORD, M.M. (1976) A rapid and sensitive method for quantitation of microgram quantities of protein, utilizing the principle of protein-dye binding. *Analyt. Biochem.* **72**, 248–254.
- CALDEYRO-BARCIA, R. & POSEIRO, J.J. (1959) Oxytocin and the contractility of the human uterus. *Ann. N.Y. Acad. Sci.* **75**, 813–830.
- CHALLIS, J.R.G., DAVIES, I.J. & RYAN, K.J. (1973) The concentration of progesterone, estrone and 17- β estradiol in the plasma of pregnant rabbits. *Endocrinology* **93**, 971–976.
- CHAN, W.Y., HILL, P.S. & HRUBY, V.J. (1990) Oxytocin- and vasopressin-binding sites in the rat uterus: competition binding and inhibitory pA₂ studies with oxytocin and oxytocin antagonists. *Endocrinology* **126**, 2095–2101.
- ELANDS, J., BARBERIS, C., JARD, S., TRIBOLLET, E., DREIFUSS, J., BANKOWSKI, K., MANNING, M. & SAWYER, W.H. (1988) ¹²⁵I-labelled d(CH₂)₅[Tyr(Me)², Thr⁴, Tyr-NH₂⁹]OVT: a selective oxytocin receptor ligand. *Eur. J. Pharm.* **147**, 197–207.
- FLINT, A.P.F., LEAT, W.M.F., SHEDDRICK, E.L. & STEWART, H.J. (1986) Stimulation of phosphoinositide hydrolysis by oxytocin and the mechanism by which oxytocin controls prostaglandin synthesis in the ovine endometrium. *Biochem. J.* **237**, 797–805.
- FUCHS, A.R. (1987) Prostaglandin F_{2a} and oxytocin interactions in ovarian and uterine function. *J. Steroid Biochem.* **27**, 1073–1080.

- Fuchs, A.R. & Fuchs, F. (1984) Endocrinology of human parturition: a review. *Br. J. Obstet. Gynaecol.* **91**, 948–967.
- Fuchs, A.R., Fuchs, F., Husslein, P., Soloff, M.S. & Fernstrom, M.J. (1982) Oxytocin receptors and human parturition: a dual role for oxytocin in the initiation of labor. *Science, NY* **215**, 1396–1398.
- Fuchs, A.R., Peryasamy, S., Alexandrova, M. & Soloff, M.S. (1983) Correlation between oxytocin receptor concentration and responsiveness to oxytocin in pregnant rat myometrium: effect of ovarian steroids. *Endocrinology* **113**, 742–749.
- Fuchs, A.R., Fuchs, F., Husslein, P. & Soloff, M.S. (1984) Oxytocin receptors in human uterus during pregnancy and parturition. *Am. J. Obstet. Gynecol.* **150**, 734–741.
- Huguenin, R.L. (1966) Synthese de la desamino-1-Orn-8-vasopressine, de la desamino-1-Phe-2-Orn-8-vasopressine, de la desamino-1-Ile-3-Orn-8-vasopressine (= desamino-1-Orn-8-oxytocine) et de la desamino-1-Phe-2-Ile-3-Orn-8-vasopressine (= desamino-1-Phe-2-Orn-8-oxytocine). *Helv. Chim. Acta* **49**, 711–725.
- Kozuka, M., Ito, T., Hirose, S., Takahashi, K. & Hagiwara, H. (1989) Endothelin induces two types of contractions of rat uterus: phasic contractions by way of voltage-dependent calcium channels and developing contractions through a second type of calcium channels. *Biochem. Biophys. Res. Commun.* **159**, 317–323.
- Kruszynski, M., Lammek, B., Manning, M., Seto, J., Haldar, J., Sawyer, W.H. (1980) [1-(β -mercapto- β , β -cyclopentamethylenepropionic acid), 2-(O-methyl-tyrosine)arginine vasopressin and [1-(β -mercapto- β , β -cyclopentamethylenepropionic acid)arginine vasopressin, two highly potent antagonists of the vasopressor response to arginine vasopressin. *J. med. Chem.* **23**, 364–368.
- Lowbridge, J., Manning, M. (1977) Synthesis and some pharmacological properties of [4-Threonine, 7-Glycine]oxytocin, [1-(1-2-hydroxy-3-mercaptopropanoic acid), 4-threonine, 7-glycine]oxytocin (hydroxy[Thr⁴, Gly⁷]oxytocin, and [7-glycine]oxytocin, peptides with high oxytocic-antidiuretic selectivity. *J. med. Chem.* **20**, 120–123.
- Maggi, M., Kassis, S., Malozowski, S., Guardabasso, V. & Rodbard, D. (1986) Identification and characterization of a vasopressin isoreceptor in porcine seminal vesicles. *Proc. natn. Acad. Sci. USA* **83**, 8824–8828.
- Maggi, M., De Rossi, M., Amenta, F., Genazzani, A.D., Rodbard, D. & Serio, M. (1988a) Similarity of vasopressin receptors in seminal vesicles and renal medulla of pigs. *J. Reprod. Fert.* **84**, 401–407.
- Maggi, M., Genazzani, A.D., Giannini, S., Torrisi, C., Baldi, E., Di Tommaso, M.R., Munson, P.J., Rodbard, D. & Serio, M. (1988b) Vasopressin and oxytocin receptors in vagina, myometrium and oviduct of rabbits. *Endocrinology* **122**, 2970–2980.
- Maggi, M., Del Carlo, P., Fantoni, G., Giannini, S., Torrisi, C., Casparis, D., Massi, G.B. & Serio, M. (1990) Human myometrium during pregnancy contains and responds to VI vasopressin receptors as well as oxytocin receptors. *J. clin. Endocr. Metab.* **70**, 1142–1154.
- Maggi, M., Vannelli, G.B., Peri, A., Brandi, M.L., Fantoni, G., Giannini, S., Torrisi, C., Guardabasso, V., Barni, T., Toscano, V., Massi, G.B. & Serio, M. (1991) Immunolocalization, binding and biological activity of endothelin in rabbit uterus: effect of ovarian steroids. *Am. J. Physiol.* **270**, 292–305.
- Meyer, H.H.D., Mittermeier, T. & Schams, D. (1988) Dynamics of oxytocin, estrogen and progesterin receptors in the bovine endometrium during the estrous cycle. *Acta endocr., Copenh.* **118**, 96–104.
- Munson, P.J. & Rodbard, D. (1980) LIGAND: a versatile computerized approach for characterization of ligand-binding systems. *Analyt. Biochem.* **107**, 220–239.
- Orlando, C., Brandi, M.L., Peri, A., Giannini, S., Fantoni, G., Calabresi, E., Serio, M. & Maggi, M. (1990) Neurohypophyseal hormone regulation of endothelin secretion from rabbit endometrial cells in primary culture. *Endocrinology* **126**, 1780–1782.
- Riemer, K.R., Goldfien, A.C., Goldfien, A. & Roberts, J.M. (1986) Rabbit uterine oxytocin receptors and in vitro contractile response: abrupt changes at term and the role of eicosanoids. *Endocrinology* **119**, 699–709.
- Roberts, J.S., McCracken, J.A., Gavagan, J.E. & Soloff, M.S. (1976) Oxytocin-stimulated release of prostaglandin F_{2a} from ovine endometrium in vitro: correlation with estrous cycle and oxytocin-receptors binding. *Endocrinology* **99**, 1107–1114.
- Schrey, M.P., Read, A.M. & Steer, P.J. (1986) Oxytocin and vasopressin stimulate inositol phosphate production in human gestational myometrium and decidua cells. *Bioscience Rep.* **6**, 613–619.
- Sheldrick, E.L. & Flint, A.P.F. (1985) Endocrine control of uterine oxytocin receptors in the ewe. *J. Endocr.* **106**, 249–258.
- Soloff, M.S. (1975) Uterine receptors for oxytocin: effects of estrogens. *Biochem. Biophys. Res. Commun.* **65**, 205–212.
- Soloff, M.S. & Fields, M.J. (1989) Changes in uterine oxytocin receptor concentrations throughout the estrous cycle of the cow. *Biol. Reprod.* **40**, 283–287.
- Soloff, M.S., Schroeder, B.T., Chakraborty, J. & Pearlmutt, A.F. (1977) Characterization of oxytocin receptors in the uterus and mammary gland. *Fedn Proc. Fedn Am. Soc. exp. Biol.* **36**, 1861–1866.
- Soloff, M.S., Alexandrova, M. & Fernstrom, M.J. (1979) Oxytocin receptors: triggers for parturition and lactation? *Science, NY* **204**, 1313–1315.
- Soloff, M.S., Fernstrom, M.A., Peryasamy, S., Soloff, S., Baldwin, S. & Wieder, M. (1983) Regulation of oxytocin receptor concentration in rat uterine explants by estrogens and progesterone. *Can. J. Biochem. Cell Biol.* **61**, 625–630.
- Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K. & Masaki, T. (1988) A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature, Lond.* **332**, 411–415.

Received 22 June 1990