J. Steroid Biochem. Molec. Biol. Vol. 40, No. 4–6, pp. 481–491, 1991
Printed in Great Britain.

0960-0760/91 $3.00 + 0.00

STEROID MODULATION OF OXYTOCIN/VASOPRESSIN RECEPTORS IN THE UTERUS

MARIO MAGGI,* GUIDO FANTONI, ALESSANDRO PERI, STEFANO GIANNINI, MARIA LUISA BRANDI, CLAUDIO ORLANDO and MARIO SERIO

Department of Clinical Physiopathology, Endocrinology Unit, University of Florence, 50134 Florence, Italy

Summary—Oxytocin (OT) and V1 vasopressin (VP) receptors are present simultaneously in several tissues, including the uterus. In myometrium these receptors mediate contractility, while in endometrium they mediate the release of other uterotonic substances as endothelin (ET). In rabbit myometrium, estrogens increase, while progesterone blunts neurohypophysial hormone receptors. However, the action of sex steroids on OT and V1 VP receptors differs in terms of the ED_{50} and maximal effect. Therefore, at parturition, only OT receptors show a dramatic rise, while V1 VP receptors do not change, suggesting a major role for OT in labor. ET is a potent stimulator of uterine activity acting through specific receptors present on myometrial cells. These receptors as well as the endometrial localization of ET are modulated by sex steroids, indicating that ET might represent a paracrine regulator of uterine activity. In humans, OT but not V1 VP receptors increase as pregnancy progresses, confirming the primary relevance of OT in timing delivery.

INTRODUCTION

The demonstration of biologically active substances present in the neural part of the pituitary gland opened almost 100 years ago, the area of neuroendocrinology [1]. Kamm *et al.* [2] termed the two major extracts of the posterior pituitary, oxytocin (OT) and vasopressin (VP). The former displayed oxytocic-galactokinetic activity, while the latter showed antidiuretic properties. The first report of the synthesis of OT was in 1953 by du Vigneaud *et al.* [3]. Shortly thereafter the same group determined the structure of arginine vasopressin [AVP] [4]. Both OT and AVP are nonapeptides with two cysteine residues forming a bridge between position 1 and 6, they differ from each other only with respect to 2 amino acids: the isoleucine and leucine in OT is replaced by phenylalanine and arginine, respectively in AVP. OT, VP and their associated carrier proteins, neurophysins, are synthesized as part of larger molecules in hypothalamic nuclei, including the supraoptic nucleus and the paraventricular nucleus [5].

Recently, the nucleotide sequences of cloned cDNA encoding AVP and OT and their neurophysins were determined [6, 7]. The similarities between the 2 neurophysins and the 2 precursors, for OT and AVP, indicate that both hormones derive from a common ancestral gene and that the production of OT and AVP is a consequence of gene duplication. Hence, the 2 neurohypophysial hormones not only show consistent structural homology in their amino acid sequences but also in that of their precursor. Nonetheless, they exert quite different physiological functions. Indeed, OT is mainly involved in the control of lactation and parturition, while VP is involved in retention of water by the kidney and vasoconstriction in the peripheral circulatory system. These different biological activities of OT and VP must be mediated by different and selective receptors for these hormones. At least three distinct classes of receptors for these hormones have been described: the OT receptor and the V1 and V2 subtype of VP receptors. Renal epithelial VP receptors, mediating the antidiuretic activity of VP, are termed the V2 VP receptors as distinguished from the V1 VP receptors found on vascular smooth muscle and liver. The V2 VP receptors are coupled to the activation of adenylate cyclase activity, while the V1 VP and the OT receptors mediate the activation of polyphosphatidylinositol hydrolysis and the increase in free cytosolic calcium; review in [8]. Beside these functional criteria the different classes of receptors for the neurohypophysial hormones

Proceedings of the VIIIth International Congress on Hormonal Steroids, The Hague, The Netherlands, 16–21 September 1990.
*To whom correspondence should be addressed.

could be characterized also using selective agonists and antagonists; review in [9]. The combined use of these pharmacological probes and second messengers measurement allowed the identification of specific receptors for neurohypophysial hormones in tissues other than their traditional targets. Indeed, the development of selective radioligands such as [^{3}H][Thr^4, Gly^7]OT [10] and [^{125}I]d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4, Orn^8,TyrNH$_2^9$]OT [11] for the OT receptors; [^{3}H]dDAVP [12] for the V2 VP receptors and [^{3}H]d(CH$_2$)$_5$TyrMeAVP [13] and [^{125}I]d-(CH$_2$)$_5$[Sar^7]AVP [14] for the V1 VP receptors greatly aids in the discrimination among the different subtypes of receptors. However, the ligand specificity of neurohypophysial hormone receptors might be different in different mammals: dVDAVP is a rather selective agonist for the rat [15] and human [16] V2 VP receptors, but shows low affinity for the V2 VP receptors present in porcine renal medulla [17, 18]. Similarly d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4,Orn^8,TyrNH$_2^9$]OT is a rather selective antagonist for the rat [11] and rabbit [9] OT receptors, but the same compound is less selective in humans [20]. Furthermore, the tissue distribution of these receptors also varies among different animal species: while in porcine seminal vesicles a high concentration of V2 VP receptors is present [18, 21] in human seminal vesicles only a low concentration of V1 VP receptors was identified [16]. Therefore, cautiousness is needed in the extrapolation of the experimental results from one animal species to another.

Generally, OT and V1 VP receptors are present simultaneously in the same tissue. In the uterus OT and V1 VP receptors were localized in both the myometrial and endometrial layer, regulating smooth muscle contraction and release of other uterotropic substances [19]. Nonetheless, despite the co-localization, these two subtypes of receptors do not share the same regulators and physiological significance.

OT AND VP RECEPTORS IN MYOMETRIUM

The myometrium of nonpregnant and pregnant mammals is maintained in a relatively quiescent state for the largest part of the reproductive life; during this time its ability to respond to contractile agonists is suppressed. Approaching parturition, the sensitivity of the myometrium to contractile substances increases and coordinated, forceful contractions begin. It is generally accepted that OT represents the main uterotonic hormone. Indeed, synthetic OT is one of the most useful drugs that is available to the obstetrician to induce or accelerate labor. However, several reports indicate that AVP shows an oxytocic action equal if not greater than that of OT [22–25].

In order to clarify if the uterotonic activity of VP is mediated by specific receptors or via the cross-reactivity of AVP at the OT receptors [26], we performed saturation and homologous competition using [^{3}H]OT and [^{3}H]AVP in the female genital tract of immature rabbits in basal condition, after different treatment with sex steroids, during pregnancy and at the time of spontaneous delivery [27]. Typical Scatchard plots, derived from homologous competition experiments performed in myometrial membranes of rabbits treated with 200 μg/kg for 4 days, are shown in Fig. 1 by continuous lines. The presence of a linear relationship for both ligands suggested an homogeneity of sites; however, we found consistent differences in the specific binding of [^{3}H]OT and [^{3}H]AVP in the different groups of animals studied and in

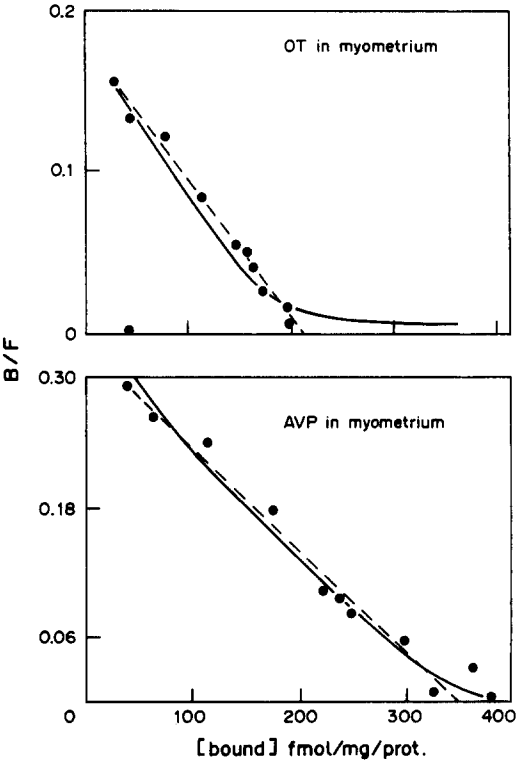

Fig. 1. Scatchard plots for [^{3}H]OT (upper panel) and [^{3}H]AVP (lower panel) derived from a typical experiment performed in the myometrium of rabbits primed with 17β-estradiol (200 μg/kg × 4 days), – – –, indicate the relationship obtained using a 1-site-model, whereas the —— indicate the relationship for a 2-sites-model. Binding experiments were performed as described in [27].

the different regions of the female genital tract (see [27] and Fig. 3). Furthermore, we observed a significant change in the order of potency of a series of ligands for the binding of [^{3}H]OT and [^{3}H]AVP to the uterus of pregnant rabbits (see [27] and Table 1.) Virtually all evidence supports the presence of an heterogeneity of sites in the female genital tract of rabbits. In order to verify the presence of distinct classes of receptors for neurohypophysial hormones we performed complete sets of self- and cross-displacement experiments between OT and AVP in uterine membranes. In addition, we decided to include in the study the 2 most selective ligands for the binding of [^{3}H]OT and [^{3}H]AVP; [Thr^4,Gly^7]OT and d(CH$_2$)$_5$TyrMeAVP. Experimental results were analyzed with the aid of computerized curve fitting (program LIGAND) [28]. The program allows for formulation and testing of several alternative models so that the most appropriate one may be chosen on the basis of an objective, statistically valid criterion, the "extra sum-of-squares" F-test. Simultaneous computer analysis was performed for 38 curves for the myometrium of rabbits pretreated with estradiol, 24 curves for the myometrium of pregnant rabbits and 19 curves for the myometrium of parturient rabbits. The results from all 3 groups clearly indicated that a 1-site model was inadequate [27]. Introduction of a 2nd independent class of sites was necessary, and sufficient, to provide satisfactory goodness of fit in all cases ($P < 0.001$ in all 3 cases). Figure 2 shows the best fit model in membranes from parturient rabbits. Similar affinity constants were obtained in the myometrium of estrogenized and pregnant rabbits (Table 2). The V1 antagonist d(CH$_2$)$_5$Tyr(Me)AVP showed selectivity for R_2 (V1 VP site), while OT and the OT agonist [Thr^4, Gly^7]OT bound preferentially to R_1 (OT site). AVP bound with high affinity to both sites. Thus, OT was rather selective for R1 whereas AVP was essentially a nonselective ligand for the 2 sites. These properties, together with the almost equal distribution of R_1 and R_2 in membranes from the myometrium of estrogenized rabbits (see Fig. 3 and Table 2), justify the apparent linearity of the Scatchard plot as in Fig. 1. Indeed computer

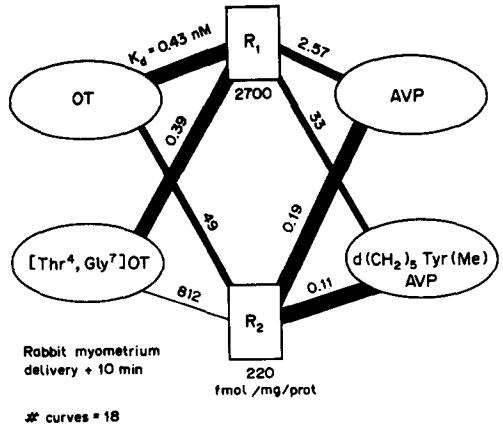

Fig. 2. Best fitting model: the relative affinities of the 4 ligands; OT, [Thr^4, Gly^7]OT, AVP and d(CH$_2$)$_5$Thr(Me)-AVP are schematically represented by the thickness of the lines connecting the ligands with the receptors. The K_d values (nM) are indicated by the numbers on each line. R_1 and R_2 denote the 2 classes of sites. The numbers below each, □, indicate the concentration of binding sites in fmol/mg/protein. Membranes were from the myometrium of rabbits at the time of spontaneous delivery. The model was fitted using 18 curves from 3 membrane preparations in 2 experiments. A model involving 2 classes of sites was significantly better than a model involving only a single class of sites ($P < 0.001$). Binding experiments were performed as described in [27].

Table 1. Rank order of potency (log IC$_{50}$) of several ligands in displacing the binding of [^{3}H]AVP and [^{3}H]OT to myometrial membranes from pregnant rabbits

Ligand	Log(IC$_{50}$) [^{3}H]AVP	Log(IC$_{50}$) [^{3}H]OT
AVP	−9.0	−8.9
OT	−7.2	−9.0
[Thr^4,Gly^7]OT	−5.7	−9.2
d(CH$_2$)$_5$TyrMeAVP	−8.9	−7.3
dPenTyrMeAVP	−8.3	−8.0
dVDAVP	−5.8	−6.7
AVT	−8.8	−9.3
d(CH$_2$)$_5$TyrEtOVT	−7.4	−7.6
Isotocin	−4.8	−4.9
dPenVDAVP	−6.8	−6.8
dGLVP	−5.1	−5.1
ASUAVT	−7.9	−8.7
TOCOH	−4.7	−5.4

Table 2. Concentration (B_{max}, fmol/mg/protein) of receptors and affinity (log K_d, pK) for OT and AVP in rabbit myometrium after priming with estradiol, during pregnancy or delivery

	OT Site	AVP Site
17β-Estradiol (200 μg/kg × 4 days)		
B_{max} (fmol/mg protein)	429 ± 93	273 ± 21
	420 ± 128	**305 ± 24**
pK OT	9.49 ± 0.02	7.46 ± 0.06
	9.39 ± 0.05	—
pK AVP	8.95 ± 0.03	9.96 ± 0.03
	—	**9.6 ± 0.1**
Pregnancy (day 29)		
B_{max}(fmol/mg/protein)	124 ± 34	416 ± 65
	174 ± 40	**457 ± 52**
pK OT	9.44 ± 0.04	7.33 ± 0.06
	9.37 ± 0.13	—
pK AVP	8.91 ± 0.07	9.56 ± 0.03
	—	**9.52 ± 0.12**
Delivery		
B_{max} (fmol/mg/protein)	2667 ± 232	233 ± 37
	2969 ± 288	**276 ± 44**
pK OT	9.37 ± 0.03	7.31 ± 0.07
	9.30 ± 0.03	—
pK AVP	8.59 ± 0.03	9.70 ± 0.03
	—	**9.60 ± 0.02**

Binding parameters (±SEM) are derived from computer analysis of families of self- and cross-displacement (1st line, roman characters) or "blocking" (2nd line, bold characters) experiments.

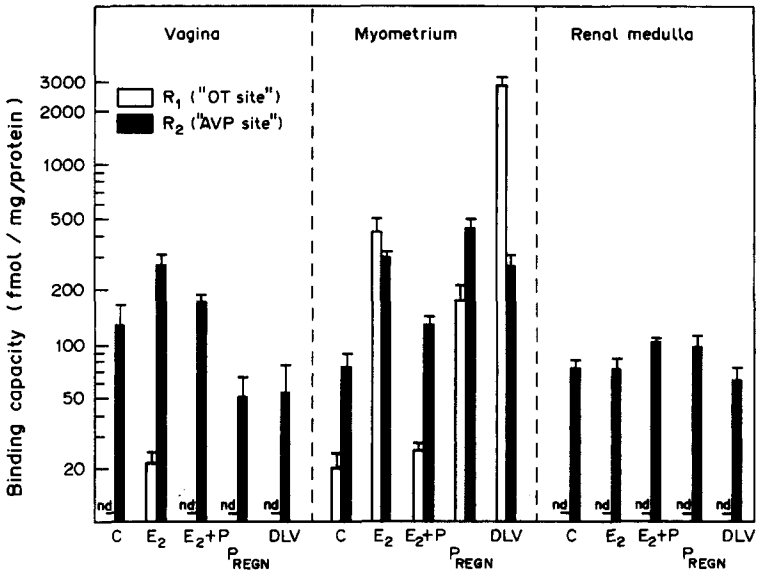

Fig. 3. Concentrations of OT (□) and AVP (■) receptors in the cervico-vaginal region, myometrium and renal medulla of rabbits. Binding capacity, fmol/mg/protein, is indicated on the ordinate using a semi-log scale. Each value of receptor concentration is the mean ± SEM of 3–6 membrane preparations. Receptor densities were derived from the analysis of "blocking" experiments for control animals (C), rabbits treated with 17β-estradiol (200 μg/kg × 4 days) (E_2) or 17β-estradiol (200 μg/kg × 4 days) followed by progesterone (5 mg/kg × 4 days) (E_2 + P). Receptor concentrations in the vagina, myometrium and renal medulla at day 29 of pregnancy (Pregn) or 5–10 min after spontaneous delivery (DLV) are also shown. Binding experiments were performed as described in [27].

modeling of such individual homologous competition curves indicated the presence of a single class of sites (Scatchard plots indicated by – – – in the figures). However, when the same experimental data were analyzed simultaneously in the context of families of self- and cross-displacement curves among OT, AVP and the 2 specific analogs, mathematical modeling strongly suggested the presence of 2 distinct populations of sites. Scatchard plots derived from the 2-sites-model are presented in Fig. 1 as continuous lines. Thus, linearity of the Scatchard plot does not really offer a relevant test for receptor homogeneity, as is sometimes claimed.

To help establish the binding capacity of the different regions of the genital tract of the female rabbit we designed multiligand experiments using homologous competition curves with [^{3}H]OT and [^{3}H]AVP in the presence of blocking concentrations of the 2 selective ligands d(CH_2)$_5$Tyr(Me)AVP (10 nM) and [Thr^4, Gly^7]OT (50 nM). This was done to mask the V1 VP and the OT sites, respectively. The results obtained using this experimental design were absolutely consistent with those derived from self- and cross-displacement curves [27] (Table 2). "Blocking" experiments are easier to perform and decrease consistently the number of experimental determinations with obvious advantage in terms of consumption of ligands and membranes.

Figure 3 illustrates the density of OT and VP receptors in the myometrium, the cervico-vaginal region and the renal medulla of the same rabbits [27]. OT receptors have a strict regional distribution, being essentially localized in the myometrium. During pregnancy the concentration of OT receptors increases, having a dramatic surge at the time of spontaneous delivery. Indeed, during delivery (day 31) a 17-fold increase above the level measured at day 29 of pregnancy was observed. VP receptors are widely distributed in the female genital tract: these receptors are present in the vagina and myometrium (Fig. 3) and also in the oviduct [27]. The density of V1 VP receptors in the uterus increases during pregnancy but does not change at the time of spontaneous delivery (Fig. 3).

In order to verify if changes in sex steroids might affect the density of OT and V1 VP receptors in the uterus we treated immature rabbits for 4 days with increasing concentrations of 17β-estradiol (2–200 μg/kg × 4 days) or with 17β-estradiol (200 μg/kg × 4 days) and progesterone (5 mg/kg × 4 days). A statistically significant, positive relationship between the concentration of estradiol and the density of both OT and V1 VP receptors in the myometrium was found ($P < 0.001$, Fig. 4).

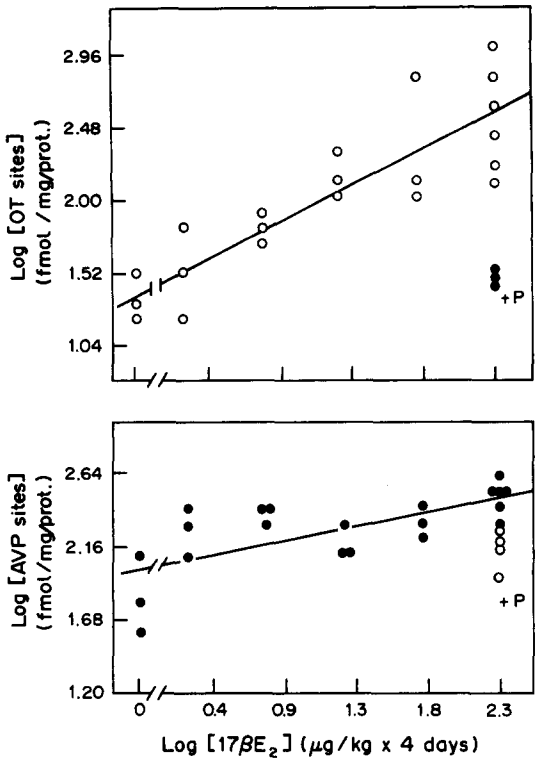

Fig. 4. Density of OT (upper panel, ○) or AVP (lower panel, ●) receptors in the myometrium of rabbits treated with increasing doses of 17β-estradiol (2–200 μg/kg × 4 days) (17βE_2). The effect of the sequential treatment with 17βE_2 (200 μg/kg × 4 days) followed by progesterone (5 mg/kg × 4 days) on OT (upper panel, ●) and AVP (lower panel, ○) receptors is also shown (+P). There is a positive statistically significant relationship ($P < 0.01$) between the logarithms of the receptor concentration and the logarithms of the dose of estrogens for both classes of receptors. Note the different effect of ovarian steroids on OT and AVP binding sites in the myometrium. Receptor densities were derived from [27].

However, while progesterone administration completely reverses the effect of estradiol on OT receptors, it was less effective on V1 VP receptors. Furthermore, while estradiol causes a 21-fold increase in the OT receptor density, the increase in the density of V1 VP receptors was less pronounced (4-fold) [27] (Fig. 4). The different sensitivity of OT and V1 VP receptors to sex steroids might therefore explain the modifications of these receptors during pregnancy and delivery: OT receptors rise during labor when progesterone falls [29]; V1 VP receptors peak during pregnancy when estradiol and progesterone increase [29].

The effect of sex steroids on neurohypophysial hormone receptors is specific for the female genital tract and not present in other target for AVP, as renal medulla (Fig. 3, Maggi M., Giannini S. and Fantoni G., unpublished observations). Although it is well accepted that during normal menstrual cycle [30, 31] or pregnancy [32, 33] there are changes in osmoregulation, our results do not support a role for renal VP receptors, at least in the rabbit.

In the rabbit, estrogen administration increases uterine contractility, while progesterone treatment has an inhibitory effect [34]. This may be relevant at the end of pregnancy, when a fall in progesterone and a rise in estrogen have been documented [29]. Even though the mechanism by which ovarian steroids influence uterine contractility is poorly understood, the regulatory influence of sex steroids on neurohypophysial hormone receptors may be crucial. Indeed, OT and V1 VP receptors are directly involved in regulating myometrial activity both in the nonpregnant [27] and pregnant uterus [20].

In humans, both OT and AVP stimulate uterine activity [20, 22–25]. Figure 5 shows the typical effect of increasing the concentration of AVP on human myometrial strips, *in vitro*, at the end of pregnancy. We found that human uterine preparations during pregnancy are continuously responsive to AVP *in vitro*, while a limited number of strips was sensitive to OT [20]. In order to explain the cellular mechanism responsible for this phenomenon we studied

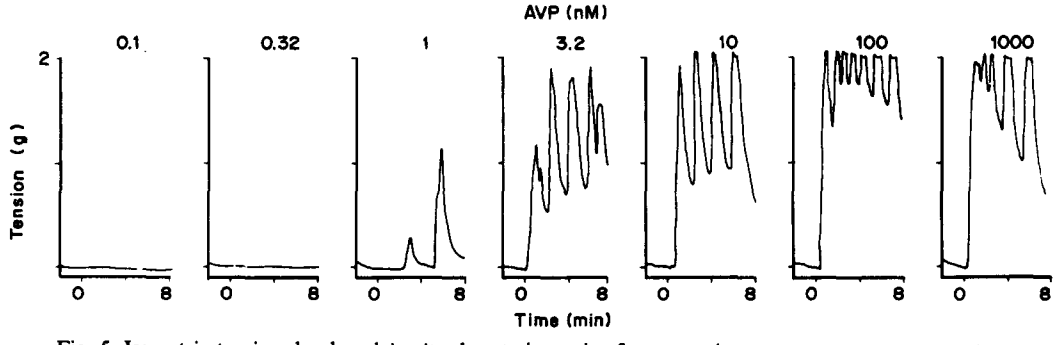

Fig. 5. Isometric tension developed *in vitro* by uterine strips from a patient at term pregnancy. At time zero increasing concentrations of AVP were added to the bath, and the contractile response was recorded for 8 min. The contractility study was performed as in [20].

the binding of neurohypophysial hormone to myometrial membranes derived from women undergoing cesarean section [20]. An experimental design similar to that described for the rabbit studies was employed. We found a close correlation between the concentration of OT sites and the response of uterine strips *in vitro* to OT stimulation. Indeed, only uterine specimens with a density of OT receptors higher than 150 fmol/mg/protein were stimulated *in vitro* by OT. Furthermore, we found a linear correlation between the concentration of OT receptors and *in vivo* uterine activity, as recorded before surgery with external tocography. According to previous studies [35, 36], a sharp increase in OT receptors was observed in human myometrium at the end of pregnancy. Conversely, we did not find any significant variation in the density of AVP sites throughout human pregnancy: consistent concentrations of V1 VP receptors (100 fmol/mg/protein) are present in the human uterus, regardless of the length of the pregnancy. These results indicate that the specific rise in OT but not VP receptors has a physiological role in initiating labor either in the rabbit or humans. However, in humans the falling progesterone-rising estrogen pattern was not clearly demonstrated [37, 38]. Therefore, the mechanism(s) involved in the activation of OT receptors in human myometrium at the end of pregnancy is still obscure. Preliminary results obtained in our laboratory on human non-pregnant myometrium indicate a lack of correlation among OT and V1 VP receptors and estrogens, while a positive relationship was paradoxically found between OT receptors and progesterone concentration (Maggi M., Magini A., Fiscella A., Giannini S., Fantoni G., Massi G. and Serio M., unpublished observations).

OT AND VP RECEPTORS IN ENDOMETRIUM

OT receptors are present not only in the ovine myometrium but also in the endometrium, where they play an essential role in regulating uterine secretion of $PGF_{2\alpha}$ [39]. In ewes, the binding characteristics of endometrial OT receptors are similar to those found in the myometrium [40]. Moreover, the density of endometrial as well as myometrial receptors are influenced by ovarian steroids [39].

OT receptors were also identified in human decidua and related to prostaglandins (PGs) production; review in [41]. The concentration of endometrial OT receptors increases in parallel with myometrial OT receptors at the end of human pregnancy and may have a physiological significance in stimulating PGs synthesis. PGs have been generally considered as key components of the complex mechanism regulating parturition. They increase during labor and promote uterine contractility and cervical ripening; review in [37] and [38].

We recently identified not only OT but also V1 VP receptors in rabbit endometrium, having ligand specificity similar to the myometrial sites [19]. Furthermore, we found parallel changes of endometrial and myometrial OT and V1 VP receptors during pregnancy and delivery in rabbits, indicating similar sensitivity to regulatory influences [19].

ENDOTHELIN IN UTERINE FUNCTION

In rabbit endometrium, but not myometrium, cells are present which show an intense immunostaining for endothelin [42]. In immature animals, the positivity for endothelin was maximal in the epithelial compartment of endometrium, while in rabbits treated with sex steroids the immunoreactivity was predominant in the stroma cells [42]. We found that both OT and V1 VP receptors mediate the release of endothelin-1 (ET-1) from rabbit endometrial cells in primary culture [43].

ET-1 is a 21 amino acid peptide recently isolated by Yanagisawa *et al.* [44]; reviews in [45], [46] and [47], from the culture medium of porcine aortal endothelial cells. This peptide is the first member of a new family of peptides, including: endothelin-2 (ET-2) and endothelin-3 (ET-3). The amino acid sequences of ET-2 and ET-3 differ from that of ET-1 by 2 and 5 amino acids, respectively. All these peptides are potent stimulators of smooth muscle cells activity with an order of potency: ET-2 > ET-1 > ET-3. The endothelin (ET) family shares close sequence homology with the sarafotoxin (SRTX) family, a group of cardiotoxic peptides isolated from the venom of the snake *Atractaspis engaddensis* [48].

Both in the rat [49] and rabbit [42] ET-1 and SRTX stimulate uterine activity, with an ED_{50} of 5–7 and 20–30 nM, respectively. The effect of ET-1 is strictly dependent on extracellular calcium and can be abolished by micromolar concentrations of L-type calcium channel blockers (Fig. 6) [42, 50]. These results, taken together, indicate that rabbit endometrium releases under control of neurohypophysial hormones uterotonic substances, such as ET-1.

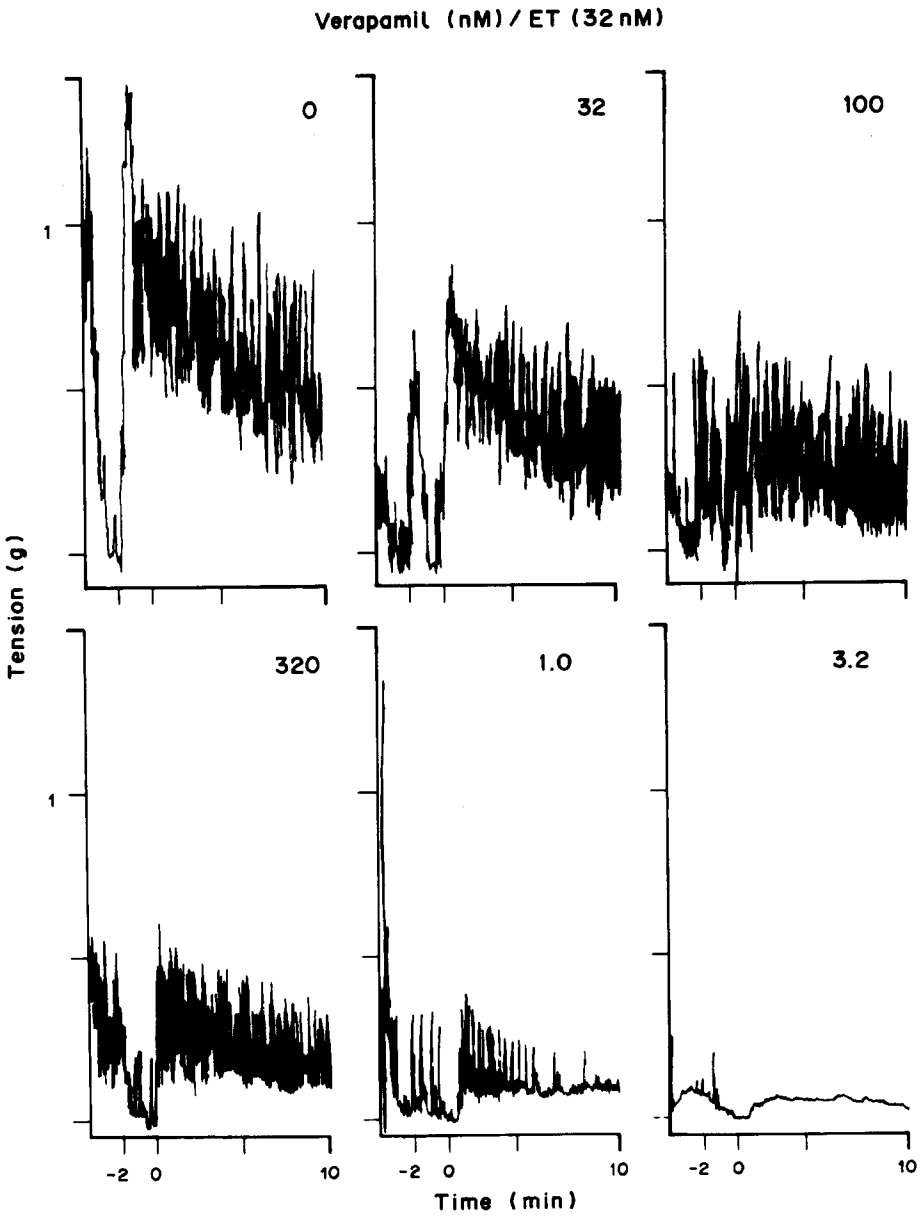

Fig. 6. Isometric tension developed *in vitro* by uterine strips of estradiol primed rabbits. At time zero the calcium antagonist verapamil was added to the bath at increasing concentrations. The contractile responses to 32 nM ET-1 administered after 2 min were recorded. In micromolar concentrations the calcium antagonist completely counteracted the contractile effect of ET-1. The contractility study was performed as in [42].

Therefore, in the uterus, ET-1 might act as a paracrine signal between endometrium and myometrium.

In order to verify this hypothesis we studied the presence of specific receptors for ET–SRTX peptides in rabbit myometrium [42]. Using labeled ET-1 and ET-3, the corresponding unlabeled peptide and SRTX we performed families of self- and cross-displacement curves. Mathematical modeling of the experimental results strongly indicates the presence of multiple receptors for ET–SRTX peptides. One site is selective for ET-1, while the second site shows approximately the same affinity for ET-1, ET-3 and SRTX. However, the former site is 10-fold more concentrated than the latter, thus explaining the enhanced biological activity of ET-1 over SRTX and ET-3 [42, 49]. Moreover, the finding of similar affinity of ET-1 for both sites justifies the apparent linearity of the Scatchard plots reported by us (Fig. 7) [42], and other laboratories; review in [47]. Although both nicardipine and verapamil antagonized the stimulatory effect of ET-1 on uterine strips,

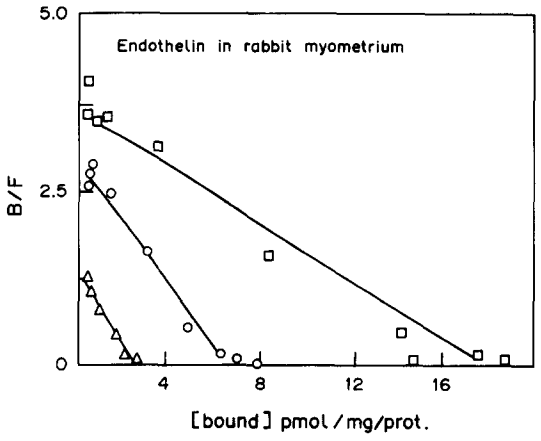

Fig. 7. Equilibrium binding of [^{125}I]ET-1 to myometrial membranes prepared from immature rabbits (△), rabbits treated with 17β-estradiol (200 μg/kg × 4 days) (E_2) (□) or 17β-estradiol (200 μg/kg × 4 days) followed by progesterone (5 mg/kg × 4 days) (E_2 + P) (○). Ordinate: B/F, bound to free ratio; abscissa: concentration of bound ligand. Experiments were carried out as in [42].

these calcium antagonists did not compete for the binding of ET-1 to myometrial membranes, indicating that in the uterus the ET receptors are distinct from the L-type calcium channels [42, 50].

Figure 7 shows that the density, but not the affinity, of ET receptors in the rabbit uterus is affected by pharmacological treatment with sex steroids. In particular, increasing concentrations of 17β-estradiol (0.2–200 μg/kg × 4 days) stimulate a dose-dependent rise in ET

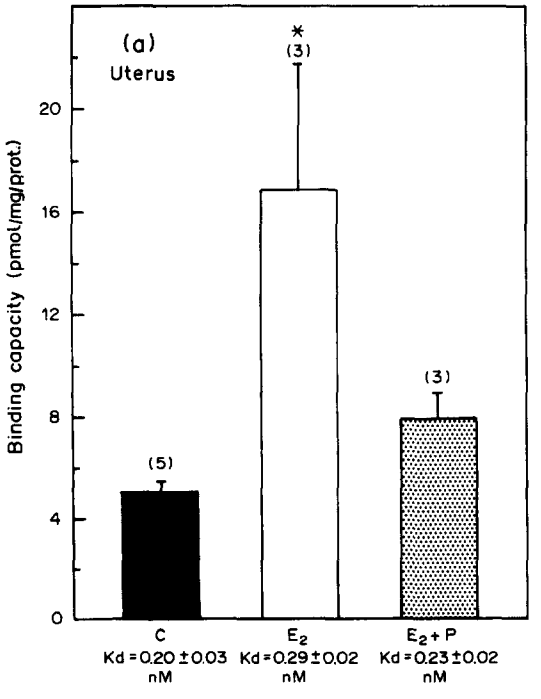

Fig. 8(a)

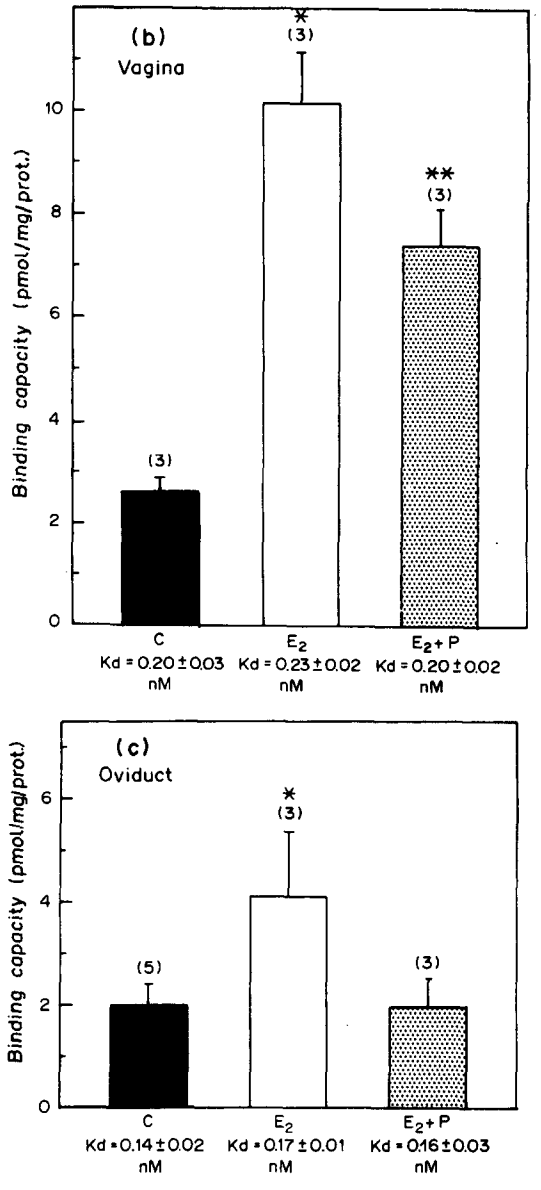

Fig. 8(b,c)

Fig. 8. Density of ET-1 receptors in the uterus (a), vagina (b) and oviduct (c) of rabbits. Binding capacity is indicated on the ordinate axis. Receptor densities were derived from the analysis of homologous competition experiments for ET-1. Control rabbits (C); rabbits treated with 17β-estradiol (200 μg/kg × 4 days) (E_2), rabbits treated as above followed by progesterone (5 mg/kg × 4 days) (E_2 + P). After treatment with estradiol, the concentration of ET-1 receptors in all the tissues investigated was significantly higher than in the C or E_2 + P treated animals (*$P < 0.05$). In the vagina, the sequential progesterone administration only partially reversed this increase (**$P < 0.05$ vs C). In contrast to the marked effect on receptor density sex steroid administration does not affect the apparent K_d. Experiments were carried out as in [42].

receptors, with an EC_{50} of 0.7 μg/kg × 4 days) [42]. The estrogen-induced increase in ET receptor concentration was completely counteracted by the sequential administration of progesterone (5 mg/kg × 4 days) [42]. This effect of

sex steroids appears to be specific to the genital tract, being present in the uterus [42], vagina and oviduct (Maggi M., Peri A., Fantoni G. and Giannini S., unpublished observations) (Fig. 8), but not in the aorta [42]. Although these results suggest that ET receptors in genital regions are induced by estrogens, with an *in vivo* system one cannot distinguish between a direct effect of sex steroids on the myometrium or an indirect effect by other estrogen-dependent substances. Hence, we studied the effect of 17β-estradiol on ET receptors using an *in vitro* system, i.e. myometrial cells in primary culture. The incubation of uterine myocytes with 1 nM 17β-estradiol for 1, 2 or 4 days did not change the density of ET receptors [42]. This result seems to indicate an indirect more than a direct effect of 17β-estradiol on ET receptors in myometrium. Preliminary results indicate that the sex steroid modulation of ET receptors is also operating during physiological events, such as pregnancy and delivery [51].

CONCLUSIONS

OT has been traditionally considered as the factor that induces labor. Ours and previous [38] studies support this concept and indicate that labor begins because of a specific and substantial increase in uterine receptors for OT with or without a rise in plasma levels of the hormone. As soon as the uterine sensitivity to OT present in maternal circulation increases enough to reach the threshold for stimulating myometrial activity, forceful and coordinated uterine contractions begin. OT facilitates the contractions of myometrium directly (myometrial receptors) or indirectly (endometrial receptors). The OT receptor interaction in the endometrium releases biologically active molecules as PGs or ET that might act on adjacent smooth muscle by increasing its tone and thereby magnifying the contractile effect of OT. In rodents, the falling progesterone-rising estrogen pattern may be responsible for the rise in OT receptors in the uterus at the time of parturition. The same changes in steroid hormones not only increase the concentration of myometrial receptors for OT, but also for other uterotonic substances as ET. In humans, the concept of progesterone withdrawal has not been substantiated [37, 38]. Moreover it is not clear which is the changing hormonal milieu of the uterus responsible for the OT receptor increase and the related myometrial activation at term pregnancy.

Even though AVP is a potent uterotonic peptide its role in parturition is not evident from studies in rodents [27, 52] or humans [20]. Receptors for AVP are continuously present and biologically active in the pregnant uterus, despite the stage of pregnancy. However, neither the concentration of AVP in maternal circulation [53] nor the density of receptors for this peptide in the uterus [20, 27, 52] increase at term pregnancy or during delivery. Elevated concentrations of AVP are secreted by the human fetus during physiological labor [54]. Furthermore, a consistent rise in fetal AVP secretion was observed in several stressful conditions [53, 55, 56]. Whether or not fetal AVP might stimulate uterine contractions is still unknown, as it is unknown which are the signals released by the fetus when the uterine residence would endanger his or maternal health.

Acknowledgements—This paper was financially supported by a grant from the University of Florence and by a grant from Centro Nazionale delle Ricerche (CNR). Dr Maggi was supported by a special grant from FIDIA Pharmaceuticals, Abano Terme, Italy.

REFERENCES

1. Oliver G. and Schafer E. A.: On the physiological action of extracts of pituitary body and certain other glandular organs. *J. Physiol.* **18** (1895) 277–279.
2. Kamm O., Aldrich T. B., Grote I. W., Rowe L. W. and Bugbee E. P.: The active principles of the posterior lobe of the pituitary gland. *J. Am. Chem. Soc.* **50** (1928) 573–583.
3. du Vigneaud V., Ressler C., Swan J. M., Roberts C. W., Katsoyannis P. G. and Gordon S.: The synthesis of an octapeptide amide with the hormonal activity of oxytocin. *J. Am. Chem. Soc.* **75** (1953) 4879–4880.
4. du Vigneaud V., Gish D. T. and Katsoyannis P. G.: A synthetic preparation possessing biological properties associated with arginine vasopressin. *J. Am. Chem. Soc.* **76** (1954) 4761–4752.
5. Sachs H. and Takabatake Y.: Evidence for a precursor in vasopressin biosynthesis. *Endocrinology* **75** (1964) 943–948.
6. Land H., Schultz G., Schmale H. and Richter D.: Nucleotide sequence of cloned cDNA encoding bovine arginine vasopressin-neurophysin II precursor. *Nature* **295** (1982) 299–303.
7. Ivell R. and Richter D.: Structure and comparison of the oxytocin and vasopressin genes from rat. *Proc. Natn. Acad. Sci. U.S.A.* **81** (1984) 2006–2010.
8. Jard S., Elands J., Schmidt A. and Barberis C. Vasopressin and oxytocin receptors: an overview. In *Progress in Endocrinology* (Edited by H. Imura, K. Shizume and S. Yoshida). Elsevier Science, Amsterdam (1988) pp. 1183–1188.
9. Sawyer W. H. and Manning M.: Experimental uses of neurohypophysial hormone analog. *TEM* 2 (1989) 48–50.
10. Elands J., Barberis C., Jard S.: [^{3}H]-[Thr^4,Gly^7]OT: a highly selective ligand for central and peripheral OT receptors. *Am. J. Physiol.* **254** (1988) E31–38.

11. Elands J., Barberis C., Jard S., Tribollet E., Dreifuss J. J., Bankowski K., Manning M. and Sawyer W. H.: ^{125}I-labeled d$(CH_2)^5$,[Tyr(Me)^2,Thr^4,Tyr-NH_2^9]OVT: a selective oxytocin receptor ligand. *Eur. J. Pharmac.* **147** (1988) 197–207.
12. Marchingo A. J., Abrahams J. M., Woodcoock E. A., Smith A. I., Mendelsohn F. A. O. and Johnston C. I.: Properties of [^{3}H]1-desamino-8-arginine vasopressin as a radioligand for vasopressin V2 receptors in rat kidney. *Endocrinology* **122** (1988) 1328–1333.
13. van Leeuwen F. W., van der Beek E. M., van Heerikhiuze J. J., Wolters P., van der Meulen G. and Wan Y.-P.: Quantitative light microscopic autoradiographic localization of binding sites labelled with [^{3}H]vasopressin antagonist d$(CH_2)_5$Tyr[Me]VP in the rat brain, pituitary, and kidney. *Neurosci. Lett.* **80** (1987) 121–125.
14. Kelly J. M., Abrahams J. M., Phillips P. A., Mendelsohn F. A. O., Grzonka Z. and Johnston C. I.: [^{125}I]-d$(CH_2)_5$[Sar^7]AVP: selective radioligand for V1 vasopressin receptors. *J. Receptor Res.* **9** (1989) 27–31.
15. Manning M., Balaspiri L., Acosta M. and Sawyer W. H.: Solid phase synthesis of [1-deamino,4-valine]-8-D-arginine-vasopressin (DVDAVP) a highly potent and specific antidiuretic agent possessing protracted effects. *J. Med. Chem.* **16** (1973) 975–978.
16. Maggi M., Baldi E., Genazzani A. D., Giannini S., Natali A., Rodbard D., Costantini A. and Serio M.: Vasopressin receptors in human seminal vesicles: identification, pharmacological characterization and comparison with the vasopressin receptors present in human kidney. *J. Andr.* **10** (1989) 393–400.
17. Butlen D., Guillon G., Rajerison R. M., Jard S., Sawyer W. H. and Manning M.: Structural requirements for activation of vasopressin-sensitive adenylate cyclase, hormone binding, and antidiuretic actions: effects of highly potent analogues and competitive inhibitors. *Molec. Pharmac.* **14** (1978) 1006–1017.
18. Maggi M., De Rossi M., Amenta F., Genazzani A. D., Rodbard D. and Serio M.: Similarity of vasopressin receptors in seminal vesicles and renal medulla of pigs. *J. Reprod. Fert.* **84** (1988) 401–407.
19. Maggi M., Peri A., Giannini S., Fantoni G. and Serio M.: Oxytocin and vasopressin receptors in rabbit endometrium during pregnancy. *J. Reprod. Fert.* **91** (1991) 575–581.
20. Maggi M., Del Carlo P., Fantoni G., Giannini S., Torrisi C., Casparis D., Massi G. B. and Serio M.: Human myometrium during pregnancy contains and responds to V1 vasopressin receptors as well as oxytocin receptors. *J. Clin. Endocr. Metab.* **70** (1990) 1142–1154.
21. Maggi M., Kassis S., Malozowski S., Guardabasso V. and Rodbard D.: Identification and characterization of a new vasopressin isoreceptor in porcine seminal vesicles. *Proc. Natn. Acad. Sci. U.S.A.* **83** (1986) 8824–8828.
22. Moir J. C.: Effect of posterior lobe pituitary gland fractions on intact human uterus. *J. Obstet. Gynaec. Br. CommW.* **51** (1944) 181–197.
23. Embrey M. P. and Moir J. C. A comparison of the oxytocic effects of synthetic vasopressin and oxytocin. *J. Obstet. Gynaec. Br. CommW.* **74** (1967) 648–652.
24. Countinho E. M. and Lopes A. C. V.: Response of the nonpregnant uterus to vasopressin as an index of ovarian function. *Am. J. Obstet. Gynec.* **102** (1968) 479–489.
25. Akerlund M. and Andersson K. E.: Vasopressin response and terbutaline inhibition on the uterus. *Obstet. Gynec.* **48** (1976) 528–36.
26. Maggi M., Malozowski S., Kassis S., Guardabasso V. and Rodbard D. Identification and characterization of two classes of receptors for oxytocin and vasopressin in porcine tunica albuginea, epididymis, and vas deferens. *Endocrinology* **120** (1987) 986–994.
27. Maggi M., Genazzani A. D., Giannini S., Torrisi C., Baldi E., Di Tommaso M., Munson P. J., Rodbard D. and Serio M. : Vasopressin and oxytocin receptors in vagina, myometrium, and oviduct of rabbits. *Endocrinology* **122** (1988) 2970–2980.
28. Munson P. J. and Rodbard D.: LIGAND a versatile computerized approach for characterization of ligand-binding system. *Analyt. Biochem.* **107** (1980) 120–139.
29. Challis J. R. G., Davies I. J. and Ryan K. J.: The concentration of progesterone, estrone and 17-β estradiol in the plasma of pregnant rabbits. *Endocrinology* **93** (1973) 971–976.
30. Spruce B. A., Baylis P. H., Burd J. and Watson M. J.: Variation in osmoregulation of arginine vasopressin during the human menstrual cycle. *Clin. Endocr.* **22** (1985) 37–42.
31. Vokes T. J., Weiss N. M., Schreiber J., Gaskill M. B. and Robertson G. L. Osmoregulation of thirst and vasopressin during normal menstrual cycle. *Am. J. Physiol.* **254** (1988) R641–647.
32. Durr J. A., Stamoutsos B. and Lindheimer M. D.: Osmoregulation during pregnancy in the rat. Evidence for resetting of the threshold for vasopressin secretion during gestation. *J. Clin. Invest.* **68** (1981) 337–346.
33. Davison J. M., Gilmore E. A., Durr J., Robertson G. L. and Lindheimer M. D.: Altered osmotic thresholds for vasopressin secretion and thirst in human pregnancy. *Am. J. Physiol.* **246** (1984) F105–109.
34. Miller M. D. and Marshall J. M.: Uterine response to nerve stimulation; relation to hormonal status and catecholamines. *Am. J. Physiol.* **209** (1965) 859–865.
35. Fuchs A. R., Fuchs F., Husslein P., Soloff M. S. and Fernstrom M. J.: Oxytocin receptors and human parturition: a dual role for oxytocin in the initiation of labor. *Science* **251** (1982) 1396–1398.
36. Fuchs A. R., Fuchs F., Husslein P. and Soloff M. S.: Oxytocin receptors in the human uterus during pregnancy and parturition. *Am. J. Obstet. Gynec.* **150** (1984) 734–741.
37. Liggins G. C., Forster C. S., Grieves S. A. and Schwartz A. L.: Control of parturition in man. *Biol. Reprod.* **16** (1977) 39–56.
38. Fuchs A. R. and Fuchs F.: Endocrinology of human parturition: a review. *Br. J. Obstet. Gynaec.* **91** (1984) 948–967.
39. Roberts J. S., McCracken J. A., Gavagan J. E. and Soloff M. S.: Oxytocin-stimulated release of prostaglandin F_{2a} from ovine endometrium *in vitro*: correlation with estrous cycle and oxytocin-receptors binding. *Endocrinology* **99** (1976) 1107–1114.
40. Ayad V. J. and Wathes D. C.: Characterization of endometrial and myometrial oxytocin receptors in the non-pregnant ewe. *J. Endocr.* **123** (1989) 11–18.
41. Fuchs A. R.: Prostaglandin F_{2a} and oxytocin interactions in ovarian and uterine function. *J. Steroid. Biochem.* **27** (1987) 1073–1080.
42. 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. and Serio M.: Immunolocalization, binding and biological activity of endothelin in rabbit uterus: effect of ovarian steroids. *Am. J. Physiol.* **260** (1991) E292–E305.
43. Orlando C., Brandi M. L., Peri A., Giannini S., Fantoni G., Calabresi E., Serio M. and Maggi M.: Neurohypophyseal hormone regulation of endothelin secretion from rabbit endometrial cells in primary culture. *Endocrinology* **126** (1990) 1780–1782.
44. Yanagisawa M., Kutihara H., Kimura S., Tomobe Y., Kobayashi M., Mitsui Y., Yazaki Y., Goto K. and Masaki T.: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* **332** (1988) 411–415.

45. Yanagisawa M. and Masaki T.: Molecular biology and biochemistry of the endothelins. *TIPS* **10** (1989) 374–378.
46. Simonson M. S. and Dunn M. J.: Endothelin pathways of transmembrane signaling. *Hypertension* **15** (Suppl. I) (1990) I-5–12.
47. Takuwa Y., Yanagisawa M., Takuwa N. and Masaki T.: Endothelin, its diverse biological activities and mechanism of action. *Prog. Growth Factor Res.* **1** (1989) 195–206.
48. Kloog Y., Ambar I., Sokolovsky M., Kochva E., Wollberg Z. and Bdolah. A.: Sarafotoxin, a novel vasoconstrictor peptide: phosphoinositide hydrolysis in rat heart and brain. *Science* **242** (1988) 268–270.
49. Bousso-Mittler D., Kloog Y., Wollberg Z., Bdolah A., Kochva E. and Sokolovsky M.: Functional endothelin/sarafotoxin receptors in the rat uterus. *Biochem. Biophys. Res. Commun.* **162** (1989) 952–957.
50. Kozuka M., Ito T., Hirose S., Takahashi K. and Hagiwara H.: 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 channel. *Biochem. Biophys. Res. Commun.* **159** (1989) 317–323.
51. Maggi M., Orlando C., Vannelli G. B., Peri A., Giannini S., Fantoni G., Barni T. and Forti G.: Oxytocin and labor: the role of endothelin. *J. Endocr. Invest.* **13** (Suppl. 1) (1990) 71.
52. Chan W. Y., Cao L., Hill P. S. and Hruby V. J.: Oxytocin- and vasopressin-binding sites in the rat uterus: competition binding and inhibitory pA_2 studies with oxytocin and oxytocin antagonists. *Endocrinology* **126** (1990) 2095–2101.
53. DeVane G. W. and Porter J. C. An apparent stress-induced release of arginine vasopressin in human neonates. *J. Clin. Endocr. Metab.* **51** (1980) 1412–1416.
54. Chard T., Hudson C. N., Edwards C. R. W. and Boyd N. H. R.: Release of oxytocin and vasopressin by the human foetus during labour. *Nature* **234** (1971) 352–354.
55. Pochard J. L. and Lutz-Bucher B.: Vasopressin and oxytocin levels in human neonates. *Acta Pediat. Scand.* **75** (1986) 774–778.
56. Hadeed A. J., Leake R. D., Weitzmann R. E. and Fisher D. A.: Possible mechanisms of high blood levels of vasopressin during the neonatal period. *J. Pediat.* **94** (1979) 805–880.