ELSEVIER

European Journal of Pharmacology 378 (1999) 137–142

ejp

www.elsevier.nl/locate/ejphar

Comparison of vasopressin binding sites in human uterine and vascular smooth muscle cells

Atsuo Tahara *, Junko Tsukada, Noe Ishii, Yuichi Tomura, Koh-ichi Wada, Toshiyuki Kusayama, Takeyuki Yatsu, Wataru Uchida, Akihiro Tanaka

Institute for Drug Discovery Research, Yamanouchi Pharmaceutical, 21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan

Received 4 March 1999; received in revised form 21 May 1999; accepted 28 May 1999

Abstract

Several studies indicate that oxytocin and vasopressin receptors in the human uterus are heterogeneous. We have investigated whether oxytocin and vasopressin bind to separate receptors or one class of receptors in human uterine smooth muscle cells. [^{3}H]d(CH_2)$_5$Tyr(Me)AVP, the vasopressin V_{1A} receptor selective radioligand, was used for comparison of vasopressin binding sites in human uterine and vascular smooth muscle cell membranes. Both membrane preparations exhibited one class of high-affinity binding sites with K_d values of 6.44 and 0.47 nM, B_{max} values of 166 and 34.8 fmol/mg protein for uterine and vascular smooth muscle cells, respectively. In vascular preparations, the selective vasopressin V_{1A} receptor antagonist, SR 49059 ((2*S*) 1-[(2*R* 3*S*)-(5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzenesulfonyl)-3-hydroxy-2,3-dihydro-1*H*-indole-2-carbonyl]-pyrrolidine-2-carboxamide), showed high affinity with K_i value of 0.98 nM, confirming that these receptors belong to the vasopressin V_{1A} receptor subtype. On the contrary, in uterine preparations, binding of [^{3}H]d(CH_2)$_5$Tyr(Me)AVP was more effectively displaced by oxytocin and the oxytocin receptor selective antagonist, L-371257, (1-{1-[4-[*N*-Acetyl-4-piperidinyl)oxy]2-methoxybenzoyl]piperidin-4-yl}-4*H*-3,1-benzoxazin-2(1*H*)-one), than vasopressin and SR 49059, suggesting that binding may be due to cross-reaction with the oxytocin receptors. These results suggest that human uterine smooth muscle cells express only a high density of oxytocin receptors.

Keywords: Oxytocin; Vasopressin; Smooth muscle cell, vascular; Smooth muscle cell, uterine

1. Introduction

The neurohypophysial nonapeptide oxytocin has a key function in controlling uterine myometrium contraction during labor (Soloff et al., 1979). In all mammalian species studied thus far, uterine sensitivity to oxytocin and oxytocin receptor numbers increase during pregnancy, reaching maximum response just prior to the onset of parturition (Caldeyro-Barcia and Sereno, 1959; Alexandrova and Soloff, 1980a,b; Maggi et al., 1990; Tence et al., 1990). This indicates the key role oxytocin plays in the initiation of labor, both before and at term. Since uterine contractions during labor can be inhibited by specific oxytocin receptor antagonists (Akerlund et al., 1987; Lopez Bernal et al., 1989; Pettibone et al., 1989; Williams et al., 1995), these antagonists are potential agents for preventing premature birth (Fuchs et al., 1982). Another neurohypophysial nonapeptide, arginine vasopressin, which differs from oxytocin by two amino acids, is involved in the regulation of vascular tone by inducting vascular smooth muscle cell contraction (Altura, 1975; Penit et al., 1983). The vascular vasopressin receptor is the vasopressin V_{1A} subtype. Furthermore, several authors have reported that vasopressin also has significant uterotonic activity (Maggi et al., 1988; Chan et al., 1990) which was apparently confirmed by the reports of separate oxytocin and vasopressin V_{1A}-like receptors in the human and rabbit uterus (Guillon et al., 1987; Ivanisevic et al., 1989; Maggi et al., 1988, 1990; Tence et al., 1990; Bossmar et al., 1994). However, oxytocin is able to displace receptor-bound [^{3}H]vasopressin and vasopressin can displace receptor-bound [^{3}H]oxytocin (Atke et al., 1995; Audugier and Barberis, 1985; Fuchs et al., 1984), leaving the question open whether oxytocin and vasopressin bind to separate uterine receptors or only one class of uterine receptors

* Corresponding author. Tel.: +81-298-54-1584; fax: +81-298-56-2558; E-mail: tahara@yamanouchi.co.jp

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00403-3

with affinity for both peptides. Previous investigations have characterized oxytocin and vasopressin receptors in rat and human uterine and mammary gland tissue, in the present study, the vasopressin binding sites in human uterine smooth muscle cells were characterized using radioligand binding assays. These data were then compared with those of vasopressin V_{1A} receptors from human vascular smooth muscle cells.

2. Materials and methods

2.1. Materials

Oxytocin and vasopressin were obtained from Peptide Institute (Osaka, Japan). [Thr^4,Gly^7]oxytocin ([4-Threonine,7-glycine]oxytocin) (Lowbridge et al., 1977), d(CH$_2$)$_5$Tyr(Me)AVP ([β-mercapto-β,β-cyclopentamethylenepropionyl^1,*O*-Me-Tyr^2, Arg^8]-Vasopressin) (Kruszynski et al., 1980) and dDAVP ([deamino-Cys^1,D-Arg^8]-Vasopressin) (Zaoral et al., 1967) were purchased from Sigma (St. Louis, MO, USA). YM087 (4′-[(2-methyl-1,4,5,6-tetrahydroimidazo[4,5-*d*][1]benzazepine-6-yl)carbonyl]-2-phenylbenzanilide monohydrochloride) (Tahara et al., 1997), SR 49059 ((2*S*) 1-[(2*R* 3*S*)-(5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzenesulfonyl)-3-hydroxy-2,3-dihydro-1*H*-indole-2-carbonyl]-pyrrolidine-2-carboxamide) (Serradeil-Le Gal et al., 1993), SR 121463A (1-[4-(*N-tert*-butyl-carbamoyl)-2-methoxybenzene sulfonyl]-5-ethoxy-3-spiro-[4-(2-morpholinoethoxy) cyclohexane]indol-2-one; equatorial isomer) (Serradeil-Le Gal et al., 1996) and L-371257 (1-{1-[4-[(*N*-Acetyl-4-piperidinyl)-oxy]-2-methoxybenzoyl] piperidin-4-yl}-4*H*-3,1-benzoxazin-2(1*H*)-one) (Williams et al., 1995) were synthesized at Yamanouchi Pharmaceutical (Ibaraki, Japan). The structures of these compounds were determined by ^{1}H-nuclear magnetic resonance, mass spectrometry and elemental analysis. Their purity was determined to be $>98\%$ by high-pressure liquid chromatography. These antagonists were initially dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10^{-2} M and diluted to the desired concentration with assay buffer. The final concentration of DMSO in the assay buffer did not exceed 1%, which did not affect [^{3}H]d(CH$_2$)$_5$Tyr(Me)AVP binding. [^{3}H]d-(CH$_2$)$_5$Tyr(Me)AVP (specific activity, 30.8 Ci/mmol) was obtained from DuPont-New England Nuclear (Boston, MA, USA). Fetal calf serum and trypsin–EDTA were from Gibco (Grand Island, NY, USA). Bovine serum albumin was from Nacalai Tesque (Kyoto, Japan). All other chemicals were of the highest reagent grade available.

2.2. Cell culture

Human uterine and vascular smooth muscle cells imported from Clonetics (San Diego, CA, USA) were purchased from IWAKI (Tokyo, Japan). The cells were grown at 37°C in Smooth Muscle Cell Basal Medium (SmBM) (Clonetics) supplemented with 0.5 μg/ml human epidermal growth factor, 5 mg/ml insulin, 1 μg/ml human fibroblast growth factor, 5% fetal calf serum and antibiotics (GA-1000) in a humidified atmosphere of 5% CO_2 in air. Cells were subcultured every 7 days into a 150 cm^2 culture dish using 0.05% trypsin–0.53 mM EDTA, and the culture medium was changed every 3 days. Cells at passage 5–10 were used for the experiment and were identified histochemically using anti-α-actin and factor VIII antibodies.

2.3. Membrane preparation

Uterine and vascular smooth muscle cells in a 500 cm^2 culture dish were washed twice with phosphate-buffered saline (PBS), scraped into a solution containing 250 mM sucrose, 10 mM $MgCl_2$ and 50 mM Tris–HCl (pH 7.4), and homogenized with a Polytron (Kinematica; Lucerne, Switzerland) at 4°C. After centrifugation at $2000 \times g$ for 10 min at 4°C, the supernatant was centrifuged at $40\,000 \times g$ for 20 min at 4°C. The resulting pellet was resuspended in 50 mM Tris–HCl (pH 7.4) containing 10 mM $MgCl_2$ and stored in small aliquots at −80°C until use. Protein was determined by the Coomassie blue method using bovine serum albumin as a standard.

2.4. [^{3}H]d(CH$_2$)$_5$Tyr(Me)AVP binding assay

For saturation binding studies, membrane preparations were incubated with various concentrations of [^{3}H]d-(CH$_2$)$_5$Tyr(Me)AVP (3–80 nM for uterine and 0.2–6.0 nM for vascular smooth muscle cell membranes). For competition studies, [^{3}H]d(CH$_2$)$_5$Tyr(Me)AVP (10 nM for uterine and 0.5 nM for vascular smooth muscle cell membranes) was added to membrane preparations, which were then incubated with various concentrations of test compound in 250 μl of assay buffer (50 mM Tris–HCl, pH 7.4, containing 10 mM $MgCl_2$ and 0.05% bovine serum albumin). Binding reactions were initiated by the addition of the membrane preparation and assay mixtures were incubated for 60 min at 30°C, which allowed equilibrium to be established. After incubation, the reaction was terminated by addition of 3 ml of ice-cold Tris buffer (50 mM Tris–HCl, pH 7.4, and 10 mM $MgCl_2$) followed immediately by rapid filtration through Whatman GF/C filters. The filters were counted with a liquid scintillation counter (Packard Tricarb scintillation counter; Packard Instrument, CT, USA). Nonspecific binding was determined in the presence of excess d(CH$_2$)$_5$Tyr(Me)AVP (1 μM). The inhibitory dissociation constant (K_i) was calculated from the following formula: $K_i = IC_{50}/(1 + [L]/K_d)$, where $[L]$ is the concentration of radioligand present in the test mixture and K_d is the dissociation constant of radioligand obtained from Scatchard plot analysis (Cheng and Prusoff,

1973). Data were analyzed using GraphPad PRISM software (GraphPAD Software, San Diego, CA, USA).

3. Results

3.1. Saturation of [^{3}H]d(CH$_2$)$_5$Tyr(Me)AVP binding

Saturation experiments using increasing concentrations of [^{3}H]d(CH$_2$)$_5$Tyr(Me)AVP showed that specific binding was saturable for both uterine and vascular smooth muscle cell membrane preparations (Fig. 1). Scatchard analysis of this data indicated the presence of one class of high affinity binding sites for both membrane preparations (Fig. 1, insets). For uterine smooth muscle cell membranes, $K_d = 6.44 \pm 0.79$ nM and $B_{max} = 166 \pm 4.64$ fmol/mg protein, while for vascular smooth muscle cell membranes, $K_d = 0.47 \pm 0.08$ nM and $B_{max} = 34.8 \pm 1.99$ fmol/mg protein. The Hill coefficient (n_H) for binding of

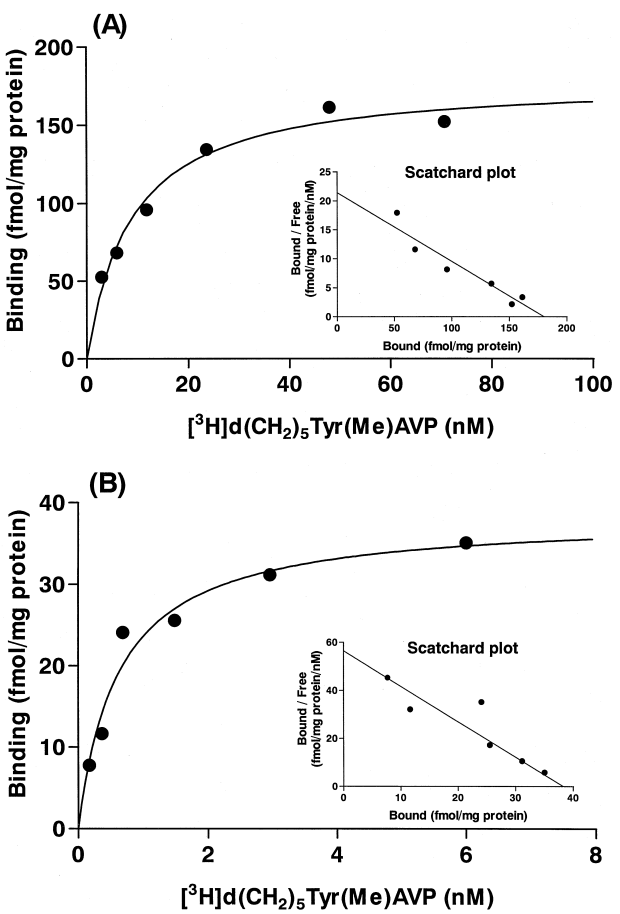

Fig. 1. Saturation equilibrium specific binding of [^{3}H]d(CH$_2$)$_5$-Tyr(Me)AVP to (A) human uterine and (B) vascular smooth muscle cell membrane preparations. Increasing concentrations of [^{3}H]d(CH$_2$)$_5$-Tyr(Me)AVP were incubated with membrane preparations (uterine: 0.1 mg protein/tube, vascular: 0.25 mg protein/tube) for 60 min at 30°C. Inset, Scatchard linear transformation of the data. Results represent data from four independent determinations performed in duplicate.

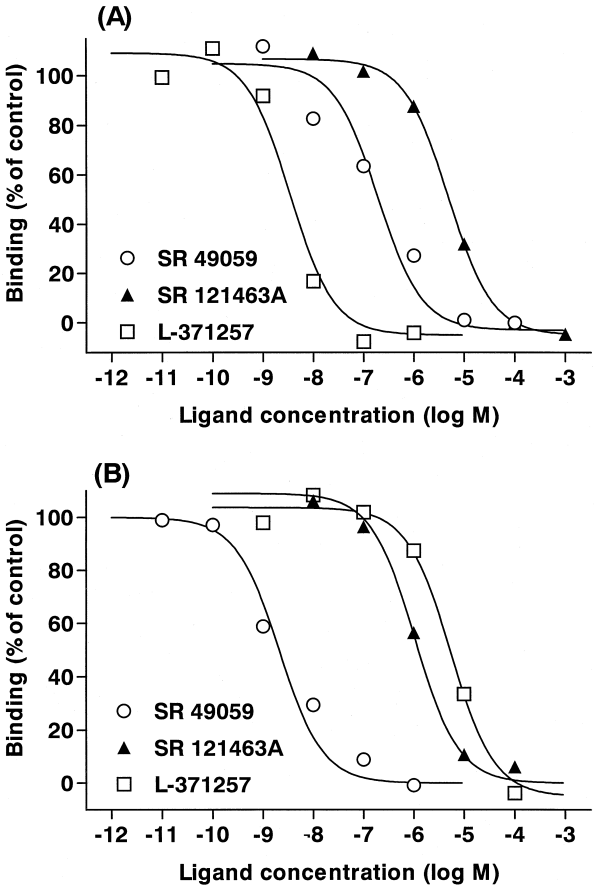

Fig. 2. Displacement of specific [^{3}H]d(CH$_2$)$_5$Tyr(Me)AVP binding to (A) human uterine and (B) vascular smooth muscle cell membrane preparations by oxytocin and vasopressin receptor ligands. Membrane preparations (uterine: 0.1 mg protein/tube, vascular: 0.25 mg protein/tube) were incubated with [^{3}H]d(CH$_2$)$_5$Tyr(Me)AVP (uterine: 10 nM, vascular: 0.5 nM) in the presence or absence of increasing concentrations of unlabelled ligands for 60 min at 30°C. Specific binding of [^{3}H]d-(CH$_2$)$_5$Tyr(Me)AVP is expressed as a percentage of control binding. Results are representative data from four independent determinations performed in duplicate.

[^{3}H]d(CH$_2$)$_5$Tyr(Me)AVP did not significantly differ from unity for either membrane preparation.

3.2. characterization of human uterine and vascular [^{3}H]d(CH$_2$)$_5$Tyr(Me)AVP binding sites

To characterize the [^{3}H]d(CH$_2$)$_5$Tyr(Me)AVP binding sites in human uterine and vascular smooth muscle cell membrane preparations, the abilities of a variety of vasopressin and oxytocin receptor agonists and antagonists to displace [^{3}H]d(CH$_2$)$_5$Tyr(Me)AVP were investigated. Typical displacement curves are shown in Fig. 2, and K_i values are summarized in Table 1. For human vascular smooth muscle cell membranes, the V_{1A} receptor selective antagonist, SR 49059, and the V_{1A}/V_2 receptor antagonist, YM087, showed high affinity with K_i values of 0.98 ± 0.18 and 5.67 ± 0.81 nM, respectively. In contrast, the V_2 receptor selective antagonist, SR 121463A, and oxytocin

Table 1
K_i values of oxytocin and vasopressin receptor ligands for human uterine and vascular smooth muscle cell membrane preparations and human oxytocin and vasopressin V_{1A} receptors
Values are mean ± S.E.M. obtained from four independent determinations performed in duplicate. Corresponding values for human oxytocin and vasopressin V_{1A} receptors are taken from previously reported data.

No.	Compound	[^{3}H]d(CH$_2$)$_5$Tyr(Me)AVP		[^{3}H]oxytocin	[^{3}H]vasopressin[e]
		Uterine smooth muscle cells	Vascular smooth muscle cells	Oxytocin receptors	Vasopressin V_{1A} receptors
1	vasopressin	5.02 ± 0.83	0.48 ± 0.08	7.0 ± 1.6[a]	0.56 ± 0.11
2	oxytocin	1.69 ± 0.04	14.9 ± 2.31	1.9 ± 0.11[a]	5.46 ± 1.97
3	d(CH$_2$)$_5$Tyr(Me)AVP	4.19 ± 0.59	0.37 ± 0.08	5.9 ± 1.0[a]	0.77 ± 0.18
4	dDAVP	154 ± 31.3	38.5 ± 11.0	140 ± 16[a]	62.4 ± 17.6
5	[Thr^4,Gly^7]oxytocin	21.1 ± 2.24	198 ± 27.2	22 ± 3.9[a]	231 ± 50.9
6	SR 49059	54.1 ± 14.6	0.98 ± 0.18	130[b]	0.53 ± 0.08
7	SR 121463A	1260 ± 217	493 ± 116	1213 ± 383[c]	304 ± 7.29
8	YM087	38.0 ± 2.84	5.67 ± 0.81		4.30 ± 0.99
9	L-371257	1.35 ± 0.25	2060 ± 259	46 ± 0.25[d]	1450 ± 641

[a] Jasper et al. (1995).
[b] Serradeil-Le Gal et al. (1993).
[c] Serradeil-Le Gal et al. (1996).
[d] Williams et al. (1995).
[e] Tahara et al. (1998).

receptor selective antagonist, L-371257, exhibited much lower affinity with K_i values of 493 ± 116 and 2060 ± 259 nM, respectively. For human uterine smooth muscle cell membranes, SR 49059, SR 121463A and YM087 showed low affinity with K_i values of 54.1 ± 14.6, 1260 ± 217 and 38.0 ± 2.84 nM, respectively, however, L-371257 exhibited high affinity with K_i value of 1.35 ± 0.25 nM. In human vascular smooth muscle cell membranes, the order of potency was d(CH$_2$)$_5$Tyr(Me)AVP = vasopressin > SR 49059 > YM087 > oxytocin > dDAVP > [Thr^4, Gly^7]oxytocin > SR 121463A > L-371257, while in human uterine smooth muscle cell membranes, L-371257 = oxytocin > d(CH$_2$)$_5$Tyr(Me)AVP = vasopressin > [Thr^4, Gly^7]oxytocin > YM087 > SR 49059 > dDAVP > SR 121463A. To put these receptor affinities in perspective, Table 1 also includes previously published data from different sources on oxytocin receptor binding affinity in membranes derived from human uterus and vasopressin V_{1A} receptor binding affinity in membranes derived from human V_{1A} receptors expressed on Chinese hamster ovary (CHO) cells. For the entire series of oxytocin and vasopressin receptor ligands tested, there was a highly correlation between the pK_i values determined on human uterine smooth muscle cell membranes and the corresponding values measured on human uterus oxytocin receptors (Fig. 3C). No such correlation was found when comparing the ligand specificity of human uterine smooth muscle cell membranes to those of human vascular smooth muscle cell and human vasopressin V_{1A} receptor-expressing CHO cell membranes (Fig. 3A,B).

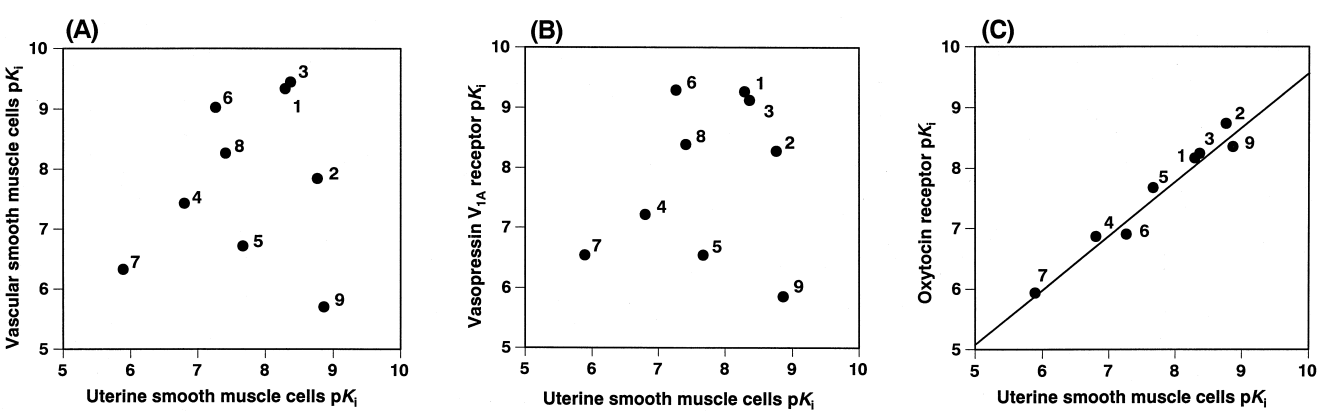

Fig. 3. Ligand selectivity of human uterine smooth muscle cell receptors; comparison with (A) human vascular smooth muscle cell, (B) human vasopressin V_{1A} and (C) human oxytocin receptors (constructed from data given in Table 1). pK_i ($-\log K_i$) values for binding of oxytocin and vasopressin receptor agonists and antagonists tested (key to symbols in legend to Table 1) to human uterine smooth muscle cells are plotted against corresponding values determined for human vascular smooth muscle cell, human vasopressin V_{1A} and oxytocin receptors.

4. Discussion

Previous investigations have demonstrated that oxytocin induces rise in intracellular Ca^{2+} concentration in rat and human uterine smooth muscle cells (Arnaudeau et al., 1994; Holda et al., 1996). However, these studies did not examine which subtype of the oxytocin–vasopressin receptor family mediates this response. It has been reported that oxytocin induces rise in intracellular Ca^{2+} concentration via the human V_{1A} receptors (Tahara et al., 1998). Recently, several newly developed antagonists with high selectivity for oxytocin or vasopressin receptor subtypes are valuable in further studying the expression and functional role of oxytocin and vasopressin binding sites in the human uterus. In the present study, the direct characterization of vasopressin and oxytocin binding sites in human uterine smooth muscle cells was performed.

The present study clearly demonstrates the existence of specific, high-affinity binding sites for $[^3H]d(CH_2)_5$-Tyr(Me)AVP in human uterine and vascular smooth muscle cells. The K_d value obtained in human vascular smooth muscle cell membrane is in good agreement with that reported for the vasopressin V_{1A} receptors (Serradeil-Le Gal et al., 1995), however, that obtained in human uterine smooth muscle cell membrane is lower than that reported for the vasopressin V_{1A} receptors. (Tahara et al., 1998) and is in agreement with that reported for the oxytocin receptor expressed in CHO cells (Thibonnier et al., 1998). Furthermore, the rank order of potency for several ligands particularly SR 49059, the vasopressin V_{1A} receptor selective antagonist, and L-371257, the oxytocin receptor selective antagonist, were markedly different between uterine and vascular smooth muscle cells. The K_i value for SR 49059 is 0.98 nM while the value for L-371257 is 2060 nM in vascular smooth muscle cells. However, the K_i value for SR 49059 is 54.1 nM and the value for L-371257 is 1.35 nM in uterine smooth muscle cells, indicating that the $[^3H]d(CH_2)_5$Tyr(Me)AVP binding sites in human uterine smooth muscle cells consist of oxytocin receptors. For the entire series of oxytocin and vasopressin receptor ligands tested, there was a highly correlation between pK_i values determined with human uterine smooth muscle cell membranes and the corresponding values measured for oxytocin receptors in human uterus ($r = 0.965$). No such correlation was found when comparing the ligand specificity of oxytocin receptors in human uterine smooth muscle cell membranes with those of vasopressin V_{1A} receptors in human vascular smooth muscle cell and human vasopressin V_{1A} receptor-expressing CHO cell membranes (r-value of 0.035 and 0.038, respectively).

To date, several lines of evidence raised the question of possible heterogeneity in uterine oxytocin and vasopressin receptors. Binding studies using tritiated oxytocin or vasopressin revealed the presence of at least two categories of sites linking oxytocin and vasopressin V_{1A} receptors in the human and rabbit uterus (Pliska et al., 1986; Guillon et al., 1987; Maggi et al., 1988, 1990; Tence et al., 1990). In contrast, Anouar et al. (1996) reported that in the rat uterus vasopressin binds to a receptor that is different from both the classical vasopressin V_{1A} subtype from vascular tissue and the classical oxytocin receptor as described in the literature, suggesting that oxytocin and vasopressin bind to a receptor that might be different from those currently characterized. Likewise, another study group recently identified separate uterotonic oxytocin receptors and decidual prostaglandin-releasing oxytocin receptors in pregnant rats (Chan et al., 1993; Chen et al., 1994). However data from the present study suggest that, unlike rat uterine tissue, human uterine smooth muscle cells do not have a specific vasopressin binding site and express only a classical oxytocin receptor. The uterine and vascular smooth muscle cells used in the present study are stable cell line and not human tissues, they cannot be compared to rat and human uterine tissues done. However, human uterine smooth muscle cells should prove useful in further characterizing the binding and biochemical function of oxytocin and its analogues, as well as aiding evaluation of human oxytocin receptor antagonists.

Acknowledgements

The authors acknowledge Drs. Toichi Takenaka, Takashi Fujikura, Noboru Satoh, Isao Yanagisawa, Gensei Kon, Yuichi Iizumi, Hisataka Shikama and Kazuo Honda (Yamanouchi Pharmaceutical) for their valuable comments and continuing encouragement.

References

Akerlund, M., Stromberg, P., Hauksson, A., Andersen, L.F., Lyndrup, J., Trojnar, J., Melin, P., 1987. Inhibition of uterine contractions of premature labor with an oxytocin analogue. Results from a pilot study. Br. J. Obstet. Gynaecol. 94, 1040–1044.

Alexandrova, M., Soloff, M.S., 1980a. Oxytocin receptors and parturition: I. Control of oxytocin concentration in the rat myometrium at term. Endocrinology 106, 730–735.

Alexandrova, M., Soloff, M.S., 1980b. Oxytocin receptors and parturition: II. Concentration of receptor for oxytocin and estrogen in the gravid and nongravid uterus at term. Endocrinology 106, 736–738.

Altura, B.M., 1975. Dose–response relationships for arginine vasopressin and synthetic analogs on three types of rat blood vessels: possible evidence for regional differences in vasopressin receptor sites within a mammal. J. Pharmacol. Exp. Ther. 193, 413–423.

Anouar, A., Clerget, M.S., Durroux, T., Barberis, C., Germain, G., 1996. Comparison of vasopressin and oxytocin receptors in the rat uterus and vascular tissue. Eur. J. Pharmacol. 308, 87–96.

Arnaudeau, S., Leprêtre, N., Mironneau, J., 1994. Oxytocin mobilizes calcium from a unique heparin-sensitive and thapsigargin-sensitive store in single myometrial cells from pregnant rats. Pflugers Arch. 428, 51–59.

Atke, A., Vilhardt, H., Hauzerova, L., Barth, T., Andersen, L.F., 1995. Effects of the non-peptide inhibitor OPC-21268 on oxytocin and vasopressin stimulation of rat and human myometrium. Eur. J. Pharmacol. 281, 63–68.

Audugier, S., Barberis, C., 1985. Pharmacological characterization of two

specific binding sites for neurohypophyseal hormones in hippocampal synaptic plasma membranes of the rat. EMBO J. 4, 1407–1412.

Bossmar, T., Akerlund, M., Fantoni, G., Szamatowicz, J., Melin, P., Maggi, M., 1994. Receptors for and myometrial response to oxytocin and vasopressin in preterm and term human pregnancy: effects of the oxytocin antagonist atosiban. Am. J. Obstet. Gynecol. 171, 1634–1642.

Caldeyro-Barcia, R., Sereno, J.A., 1959. The response of the human uterus to oxytocin throughout pregnancy, in: Caldeyro-Barcia, R., Heller, H. (Eds.), Oxytocin, Pergamon Press, London, p. 177.

Chan, W.Y., Cao, L., 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.

Chan, W.Y., Chen, D.L., Manning, M., 1993. Oxytocin receptor subtypes in the pregnant rat myometrium and decidua: pharmacological differentiations. Endocrinology 132, 1381–1386.

Chen, D.L., Chan, W.Y., Manning, M., 1994. Agonist and antagonist specificities of decidual prostaglandin-releasing oxytocin receptors and myometrial uterotonic oxytocin receptors in pregnant rats. J. Reprod. Fertil. 102, 337–343.

Cheng, Y., Prusoff, W.H., 1973. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (IC_{50}) of an enzymatic reaction. Biochem. Pharmacol. 22, 3099–3108.

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 215, 1396–1398.

Fuchs, A.R., Fuchs, F., Husslein, P., Soloff, M.S., 1984. Oxytocin receptors in the human uterus during pregnancy and parturition. Am. J. Obstet. Gynecol. 150, 734–741.

Guillon, G., Balestre, M.N., Roberts, J.M., Bottari, S.P., 1987. Oxytocin and vasopressin: distinct receptors in myometrium. J. Clin. Endocrinol. Metab. 64, 1129–1135.

Holda, J.R., Oberti, C., Perez-Reyes, E., Blatter, L.A., 1996. Characterization of an oxytocin-induced rise in $[Ca^{2+}]_i$ in single human myometrium smooth muscle cells. Cell Calcium 20, 43–51.

Ivanisevic, M., Behrens, O., Helmer, H., Demarest, K., Fuchs, A.R., 1989. Vasopressin receptors in human pregnant myometrium and decidua: interactions with oxytocin and vasopressin agonists and antagonists. Am. J. Obstet. Gynecol. 161, 1637–1643.

Jasper, J.R., Harrell, C.M., O'Brein, J.A., Pettibone, D.J., 1995. Characterization of the human oxytocin receptor stably expressed in 293 human embryonic kidney cells. Life Sci. 57, 2253–2261.

Kruszynski, M., Lammek, B., Manning, M., Seto, J., Haldar, J., Sawyer, W.H., 1980. [1-(Beta-mercapto-beta,beta-cyclopentamethylenepropionicacid),2-(*O*-methyl) tyrosine]arginine–vasopressin and [1-(beta-mercapto-beta,beta-cyclopenta methylenepropionic acid)]arginine–vasopressin, two highly potent antagonists of the vasopressor response to arginine–vasopressin. J. Med. Chem. 23, 364–368.

Lopez Bernal, A., Phipps, S.L., Rosevear, S.K., Turnbull, A.C., 1989. Mechanism of action of the oxytocin antagonist 1-deamino-2-D-Tyr-(OEt)-4-Thr-8-Orn-oxytocin. Br. J. Obstet. Gynaecol. 96, 1108–1110.

Lowbridge, J., Manning, M., Haldar, J., Sawyer, W.H., 1977. Synthesis and some pharmacological properties of [4-threonine,7-glycine]oxytocin, [1-(L-2-hydroxy-3-mercaptopropanoic acid), 4-threonine,7-glycine]oxytocin (hydroxy[Thr^4,Gly^7]-oxytocin, and [7-glycine]oxytocin, peptide with high oxytocic–antidiuretic selectivity. J. Med. Chem. 20, 120–123.

Maggi, M., Genazzani, A.D., Giannini, S., Torrisi, C., Baldi, E., di Tomaso, M., Munson, P.J., Rodbard, D., Serio, M., 1988. 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., Serio, M., 1990. Human myometrium during pregnancy contains and responds to V1 vasopressin receptors as well as oxytocin receptors. J. Clin. Endocrinol. Metab. 70, 1142–1154.

Penit, J., Faure, M., Jard, S., 1983. Vasopressin and angiotensin II receptors in rat aortic smooth muscle cells in culture. Am. J. Physiol. 244, E72–E82.

Pettibone, D.J., Clineschmidt, B.V., Anderson, P.S., Freidinger, R.M., Lundell, G.F., Koupal, L.R., Schwartz, C.D., Williamson, J.M., Goetz, M.A., Hensens, O.D., Liesch, C.D., Springer, J.P., 1989. A structurally unique, potent, and selective oxytocin antagonist derived from *Streptomyces silvensis*. Endocrinology 125, 217–222.

Pliska, V., Heiniger, J., Müller-Lhotsky, A., Pliska, P., Ekberg, B., 1986. Binding of oxytocin to uterine cells in vitro: occurrence of several binding site populations and reidentification of oxytocin receptors. J. Biol. Chem. 261, 16984–16989.

Serradeil-Le Gal, C., Wagnon, J., Garcia, C., Lacour, C., Guiraudou, P., Christophe, B., Villanova, G., Nisato, D., Maffrand, J.P., Le Fur, G., Guillon, G., Cantau, B., Barberis, C., Trueba, M., Ala, Y., Jard, S., 1993. Biochemical and pharmacological properties of SR 49059, a new, potent, nonpeptide antagonist of rat and human vasopressin V_{1a} receptors. J. Clin. Invest. 92, 224–231.

Serradeil-Le Gal, C., Herbert, J.M., Delisee, C., Schaeffer, P., Raufaste, D., Garcia, C., Dol, F., Marty, E., Maffrand, J.P., Le Fur, G., 1995. Effect of SR 49059, a vasopressin V_{1a} antagonist, on human vascular smooth muscle cells. Am. J. Physiol. 268, H404–H410.

Serradeil-Le Gal, C., Lacour, C., Valette, G., Garcia, G., Foulon, L., Galindo, G., Bankir, L., Pouzet, B., Guillon, G., Barberis, C., Chicot, D., Jard, S., Vilain, P., Garcia, C., Marty, E., Raufaste, D., Brossard, G., Nisato, D., Maffrand, J.P., Le Fur, G., 1996. Characterization of SR 121463A, a highly potent and selective, orally active vasopressin V_2 receptor antagonist. J. Clin. Invest. 98, 2729–2738.

Soloff, M.S., Alexandrova, M., Fernstrom, M.J., 1979. Oxytocin receptors: triggers for parturition and lactation?. Science 204, 1313–1315.

Tahara, A., Tomura, Y., Wada, K., Kusayama, T., Tsukada, J., Takanashi, M., Yatsu, T., Uchida, W., Tanaka, A., 1997. Pharmacological profile of YM087, a novel potent nonpeptide vasopressin V_{1A} and V_2 receptor antagonist, in vitro and in vivo. J. Pharmacol. Exp. Ther. 282, 301–308.

Tahara, A., Saito, M., Sugimoto, T., Tomura, Y., Wada, K., Kusayama, T., Tsukada, J., Ishii, N., Yatsu, T., Uchida, W., Tanaka, A., 1998. Pharmacological characterization of the human vasopressin receptor subtypes stably expressed in Chinese hamster ovary cells. Br. J. Pharmacol. 125, 1463–1470.

Tence, M., Guillon, G., Bottari, S., Jard, S., 1990. Labelling of vasopressin and oxytocin receptors from the human uterus. Eur. J. Pharmacol. 191, 427–436.

Thibonnier, M., Conarty, D.M., Preston, J.A., Wilkens, P.L., Berti-Mattera, L.N., Mattera, R., 1998. Molecular pharmacology of human vasopressin receptors. In: Zigg, H., Bourque, C.W., Bichet, D. (Eds.), Vasopressin and Oxytocin: Molecular, Cellular, and Clinical Advances, Vol. 449. Plenum, New York, pp. 251–276.

Williams, P.D., Clineschmidt, B.V., Erb, J.M., Freidinger, R.M., Guidotti, M.T., Lis, E.V., Pawluczyk, J.M., Pettibone, D.J., Reiss, D.R., Veber, D.F., Woyden, C.J., 1995. 1-(1-[4-[*N*-acetyl-4-piperidinyl]oxy]-2-methoxybenzoyl)piperidin-4-yl)-4*H*-3,1-benzoxazin-2(1*H*)-one (L-371,257): a new, orally bioavailable, non-peptide oxytocin antagonist. J. Med. Chem. 38, 4634–4636.

Zaoral, M., Kolc, J., Sorm, F., 1967. Amino acids and peptides: LXXII. Synthesis of 1-deamino-8-D-gamma-aminobutyrine-vasopressin, 1-deamino-8-D-lysin-vasopressin, and 1-deamino-8-D-arginine-vasopressin. Coll. Czech. Chem. Commun. 32, 1250–1257.