Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 510 (2005) 9–16

www.elsevier.com/locate/ejphar

Binding domains of the oxytocin receptor for the selective oxytocin receptor antagonist barusiban in comparison to the agonists oxytocin and carbetocin

Gerald Gimpl[a,*], Rolf Postina[a], Falk Fahrenholz[a], Torsten Reinheimer[b]

[a]*Institut für Biochemie, Becherweg 30, 55099 Mainz, Germany*
[b]*Department of Non-Clinical Development, Ferring Pharmaceuticals A/S, Kay Fiskers Plads 11, 2300 Copenhagen S, Denmark*

Received 3 January 2005; accepted 6 January 2005

Abstract

We have analyzed binding domains of the oxytocin receptor for barusiban, a highly selective oxytocin receptor antagonist, in comparison to the combined vasopressin V_{1A}/oxytocin receptor antagonist atosiban and the agonists oxytocin and carbetocin. For this purpose, chimeric 'gain-in function' oxytocin/vasopressin V_2 receptors were expressed in COS-7 cells. These recombinant receptors have been produced by transfer of domains from the oxytocin receptor into the related vasopressin V_2 receptor and have already been successfully employed for the identification of ligand binding domains at the oxytocin receptor (Postina, R., Kojro, E., Fahrenholz, F., 1996. Separate agonist and peptide antagonist binding sites of the oxytocin receptor defined by their transfer into the V2 vasopressin receptor. J. Biol. Chem. 271, 31593–31601). In displacement studies with 10 chimeric receptor constructs, the binding profile of barusiban was compared with the binding profiles of the ligands oxytocin, [Arg^8]vasopressin, carbetocin, and atosiban. The binding profiles for the agonists oxytocin and carbetocin were found to be similar. For both agonists, important binding domains were the extracellular N-terminus (=E1) and the extracellular loops E2 and E3 from the oxytocin receptor. For the vasopressin V_{1A}/oxytocin receptor antagonist atosiban, none of the receptor constructs were able to provide a binding with higher affinity than the starting vasopressin V_2 receptor. In contrast, the binding of barusiban was significantly improved when the transmembrane domains 1 and 2 were transferred from the oxytocin receptor to the vasopressin V_2 receptor. The binding domain of barusiban differs from the binding domain of the agonists and the nonselective oxytocin receptor antagonist $d(CH_2)_5$[Tyr-$(Me)^2$,Thr^4,Orn^8,Tyr^9]vasotocin that has been used in previous studies. Overall, the data supported the concept of a central pocket site within the oxytocin receptor.

Keywords: Atosiban; Barusiban; Carbetocin; G-protein coupled receptors; Oxytocin

1. Introduction

Oxytocin is a neurohypophyseal nonapeptide hormone. Its major physiological role is to induce contraction of mammary myoepithelium and uterine smooth muscle. As one of the strongest uterotonic agents known, oxytocin is widely used to induce and/or augment labor. Its action is mediated by the G protein coupled oxytocin receptor that is dramatically upregulated in the myometrium immediately before parturition (Gimpl and Fahrenholz, 2001). Oxytocin receptor antagonists are, on the other hand, valuable substances for the treatment of preterm labor by reducing myometrial contractions. One such substance is atosiban. This oxytocin receptor antagonist was shown to reduce the contraction rate in preterm labor and is now used clinically for the indication of delaying imminent preterm birth. However, atosiban suffers from lack of specificity, e.g. it also binds to the vasopressin V_{1A} receptor (Akerlund et al., 1999). Recently, a much more selective oxytocin receptor

* Corresponding author. Tel.: +49 6131 3923829; fax: +49 6131 3925348.
E-mail address: gimpl@uni-mainz.de (G. Gimpl).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.01.010

antagonist, barusiban, has been developed (Nilsson et al., 2003). Barusiban shows several times higher affinity and potency for the human oxytocin receptor than vasopressin and any other oxytocin receptor antagonist (Pierzynski et al., 2004).

To understand the function of any receptor at the molecular level, the corresponding ligand binding domains need to be identified. In the case of the oxytocin receptor, the extracellular amino-terminus (Postina et al., 1996; Hawtin et al., 2001) and extracellular loop regions (Postina et al., 1996) have been shown to be involved in oxytocin binding. In particular, Arg34 within the amino-terminus of the receptor is required for high-affinity agonist binding (Wesley et al., 2002). It was suggested by molecular modelling that the oxytocin binding site is formed by the upper parts of transmembrane helices 3 to 7 plus the first and second extracellular loops (Fanelli et al., 1999). With respect to the identification of binding domains for oxytocin receptor antagonists, a more complex picture has emerged (Elands et al., 1988; Postina et al., 1996; Breton et al., 2001; Wesley et al., 2002). In studies with chimeric oxytocin/vasopressin V_2 receptor constructs, the competitive peptide receptor antagonist $d(CH_2)_5$[Tyr(Me)^2,Thr^4,Orn^8,Tyr^9] vasotocin (Elands et al., 1988) has been observed to bind at the transmembrane helices 1, 2, and 7 with a major contribution to binding affinity by the upper part of helix 7, separate from the agonist-binding domain (Postina et al., 1996). In contrast, using a iodinated photoreactive analogue of $d(CH_2)_5$[Tyr-(Me)^2,Thr^4,Orn^8,Tyr^9]vasotocin in a photoaffinity labelling approach, a tripeptide motif in the upper part of transmembrane domain 3 has been shown to be an antagonist binding site (Breton et al., 2001). Previously, $d(CH_2)_5$[Tyr-(Me)^2,Thr^4,Orn^8,Tyr^9]vasotocin has been widely used as an receptor antagonist. However, it exhibits substantial vasopressin V_{1A} antagonism (Manning et al., 1995). Therefore, the results obtained with this compound are difficult to interpret in terms of receptor specificity. This is, for example, demonstrated by the fact that the homologue tripeptide motif within the vasopressin V_{1A} receptor has also been shown to be a binding site for the photoreactive $d(CH_2)_5$[Tyr-(Me)^2,Thr^4,Orn^8,Tyr^9]vasotocin analogue (Breton et al., 2001).

This prompted us to evaluate the antagonist binding domain of the oxytocin receptor using barusiban, a highly selective oxytocin receptor antagonist. Furthermore, we explored the binding domain of carbetocin, an agonist with high stability and a long duration of action (Engstrom et al., 1998). For this purpose, we used a variety of chimeric 'gain-in function' oxytocin/vasopressin V_2 receptor constructs that were transiently expressed in COS cells. These receptors have been created by transfer of domains from the oxytocin receptor into the related vasopressin V_2 receptor and have already been successfully employed for the identification of ligand binding domains at the oxytocin receptor (Postina et al., 1996). The vasopressin V_2 receptor has been used because it shows 40% overall sequence identity to the oxytocin receptor, but it can strongly discriminate between the ligands [Arg^8]vasopressin and oxytocin; [Arg^8]vasopressin is bound with nearly 400-fold higher affinity than oxytocin. In contrast, the oxytocin receptor binds oxytocin and [Arg^8]vasopressin with similar high affinity; oxytocin is bound with only about 7–10-fold higher affinity than [Arg^8]vasopressin (Gorbulev et al., 1993; Akerlund et al., 1999). It was also investigated to what extent the introduction of oxytocin receptor domains into the vasopressin V_2 receptor changes the affinity for the following ligands: barusiban, atosiban, carbetocin, vasopressin, and oxytocin.

2. Materials and methods

2.1. Materials

The following substances were supplied by Ferring Pharmaceuticals A/S (Copenhagen): atosiban (TRACTOCILE®), barusiban (=FE200440), carbetocin (DURATOCIN®), and the radioligand [^{3}]barusiban ([5-^{3}H-D-Trp]^2-FE200440). Oxytocin and [Arg^8]vasopressin were from Bachem (Switzerland). [^{3}H][Arg^8]vasopressin was from Perkin Elmer Life Science (Mechelen, Belgium). All other substances were supplied by Sigma (Deisenhofen, Germany).

2.2. Mutagenesis

The construction of the chimeric oxytocin/vasopressin V_2 receptors and the corresponding expression vectors have been described (Postina et al., 1996). The schemata for the different receptor chimera are shown in Fig. 1B.

2.3. Cell culture, transfection, and membrane preparation

COS-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% (vol/vol) fetal calf serum. Subconfluent COS-7 cells were grown in 10 cm petri dishes and were transfected with the different expression plasmids by the DEAE-dextran method. Membrane preparations were performed as described elsewhere (Ufer et al., 1995). Cell membranes were washed and homogenized in 20 mM HEPES, pH 7.4. The protein content of the membranes was determined by the method of Bradford (Bradford, 1976) using bovine serum albumin as standard. The prepared membranes were used for the radioligand binding assays.

2.4. Radioligand binding assay

Saturation and displacement assays were performed using [^{3}H][Arg^8]vasopressin or [^{3}H]barusiban as radiolabelled ligand. The composition of the binding buffer was 50 mM HEPES, pH 7.5, 5 mM $MgCl_2$. The membranes were incubated for 30 min at 30°C. Bound and free

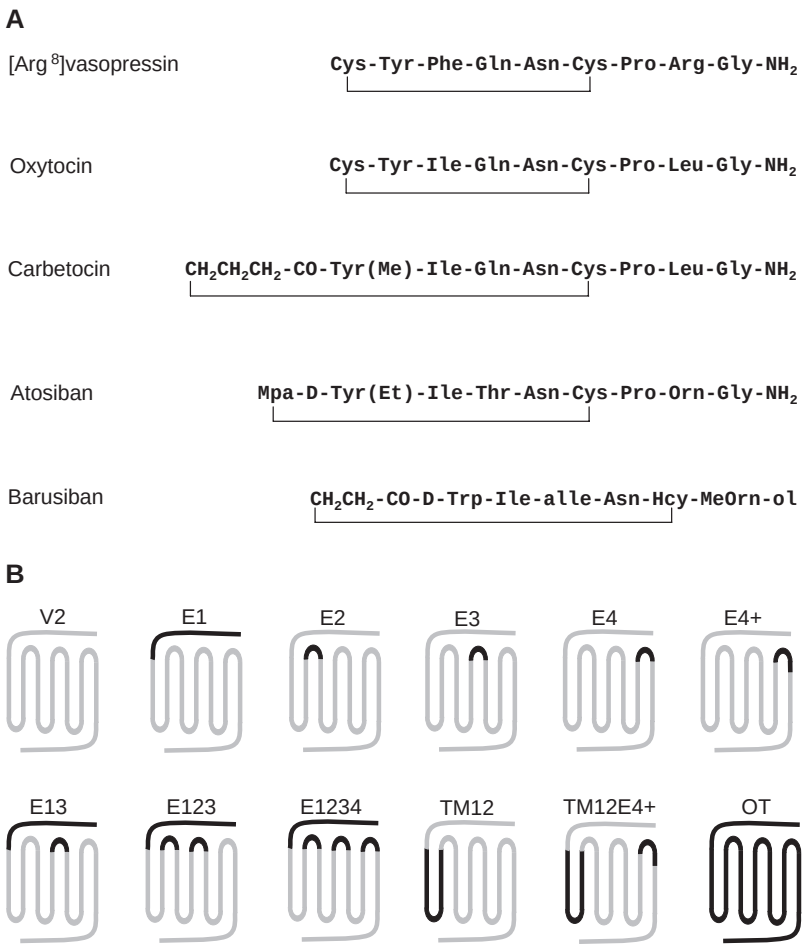

Fig. 1. Structure of oxytocin analogues (A) and schematic representation of the wild-type and chimeric receptors (B) used in this study. Abbreviations in A: alle, alloisoleucine; Hcy, homocysteine; MeOrn-ol, N^{α}-methylornithinol; Mpa, 3-mercaptopropionic acid; Orn, ornithine. Abbreviations in B: V2, vasopressin V_2 receptor; E, extracellular; TM, transmembrane; OT, oxytocin receptor.

radioactivity was separated by filtration through Whatman GF/C filters presoaked in filtration buffer (15 mM Tris/HCl, pH 7.5, at 4 °C, 2 mM $MgCl_2$) by using a filtration device (Brandel harvester).

Competition experiments were performed for all receptor constructs. For competition experiments, 5–50 μg of membrane protein was used (depending on the expression level of the constructs in COS cells). The amount of membrane protein in the assay was chosen to bind not more than 10% of the radiolabel in the test. Competition binding of 3 nM [^{3}H][Arg^8]vasopressin with unlabelled hormones in a concentration range from 0.03 nM to 100 μM was performed with barusiban as well as with the ligands [Arg^8]vasopressin, oxytocin, carbetocin, and atosiban. Non-specific binding was determined in the presence of 3 μM unlabeled [Arg^8]vasopressin. K_i values were calculated from the IC_{50} values using the Cheng–Prusoff equation: $K_i = IC_{50}/(1+[L]/K_D)$. The binding of [^{3}H]barusiban to the various oxytocin/vasopressin V_2 receptors (except for the oxytocin receptor) was of low affinity. So, saturation of the chimeric receptors with this radioligand could not be achieved even with high [^{3}H]barusiban concentrations. Under these conditions, competition of [^{3}H][Arg^8]vasopressin binding with increasing concentrations of unlabeled ligands was the method of choice.

In the case of the oxytocin receptor and the vasopressin V_2 receptor, saturation experiments were performed with [^{3}H]barusiban and [^{3}H][Arg^8]vasopressin, respectively. In saturation experiments, 5–50 μg of membrane protein and [^{3}H]barusiban or [^{3}H][Arg^8]vasopressin at concentrations from 0.1 to 20 nM were used. Nonspecific binding was determined in the presence of a 500-fold excess of the corresponding unlabeled ligand barusiban or [Arg^8]vasopressin.

All binding data were analyzed with nonlinear fitting programs (GraphPad, version 5.5). Presentation of the data was performed with GraphPad and Sigmaplot, version 8.0 (Jandel Scientific).

3. Results

The oxytocin/vasopressin V_2 receptor chimeras were transiently expressed in COS-7 cells and their membranes were analyzed with respect to their affinity for barusiban

and other oxytocin-related peptides using [^{3}H][Arg^8]vasopressin as radioligand. The structural formulae of the different peptides are shown in Fig. 1A. The results of the displacement experiments are demonstrated in Table 1 and Fig. 2. Saturation experiments using [^{3}H][Arg^8]vasopressin and [^{3}H]barusiban as radioligand verified the data calculated from competition experiments. Saturation of [^{3}H][Arg^8]vasopressin binding to the vasopressin V_2 receptor yielded a K_D value of 0.8±0.2 nM, in good agreement with the data obtained from competition experiments (K_i=0.6±0.1 nM, see Table 1). For the oxytocin receptor, the calculated K_D with [^{3}H]barusiban was 0.94±0.15 nM, in agreement with the data obtained from competition experiments (K_i=0.8±0.2 nM, see Table 1).

As expected, the ligand [Arg^8]vasopressin revealed high-affinity binding to all of the receptor constructs. Only the chimeric receptors E123 and E1234 showed slightly less affinity to [Arg^8]vasopressin. Oxytocin bound with significantly higher affinity to the vasopressin V_2 receptor when the extracellular amino-terminal domain of the oxytocin receptor (=E1) had been introduced into the vasopressin V_2 receptor. The additional transfer of extracellular domain 3 (i.e. receptors E13, E123, and E1234) further increased the affinity to oxytocin about 2-fold. Thus, the binding behavior of the ligands [Arg^8]vasopressin and oxytocin to the receptor chimeras was confirmed to be as described previously (Postina et al., 1996).

Compared with oxytocin, carbetocin is an agonist with about 10-fold lower affinity for the oxytocin receptor but with significantly higher stability and a longer duration of action (Engstrom et al., 1998). With respect to its affinity to the receptor constructs, carbetocin behaves similarly to oxytocin. Carbetocin had also higher affinity to the chimeric E1 receptor and especially to each of the combinations of E1 with the other extracellular domains, i.e. chimeric receptors E13, E123, and E1234. Notably, the transfer of the extracellular loop E3 into the E1 chimeric receptor (i.e. construct E13) led to a 90-fold increase in carbetocin binding. Thus, the extracellular loop E3 contributes significantly to the affinity of carbetocin. The transfer of the whole E2 loop into the vasopressin V_2 receptor (construct E2) did not lead to any increase in affinity for carbetocin.

Atosiban is a combined vasopressin V_{1A}/oxytocin receptor antagonist in contrast to barusiban. It also shows substantial affinity for the vasopressin V_2 receptor; the binding of atosiban to the porcine oxytocin receptor was only 7-fold higher than to the porcine vasopressin V_2 receptor (the corresponding factors for oxytocin, carbetocin, and barusiban are: 440-fold, 800-fold, and 7870-fold, respectively). None of the domains of the oxytocin receptor that have been introduced into the vasopressin V_2 receptor led to a significant improvement in atosiban's binding affinity. Even the extracellular domains that represent binding sites for the agonists oxytocin and carbetocin did not show any increased affinity for atosiban.

Barusiban, the high-affinity oxytocin receptor antagonist, discriminates substantially better than other known receptor antagonists (such as atosiban) between the oxytocin receptor and the related vasopressin V_{1A} and vasopressin V_2 receptors. This was also the case for the porcine oxytocin versus vasopressin V_2 receptor used in this study. Barusiban showed a very low affinity to the vasopressin V_2 receptor and to most of its chimeric constructs with two notable exceptions: TM12 and TM12E4+. Since the oxytocin receptor antagonist barusiban did neither bind to domain E4+ nor preferentially bind to TM12E4+ rather than to TM12, one can conclude that the transmembrane domains 1 and 2 of the oxytocin receptor should directly interact with barusiban.

4. Discussion

Several approaches have been employed to define ligand binding sites of the oxytocin receptor: site-directed muta-

Table 1
Ligand binding affinities of wild-type and chimeric receptors

Receptor	Ligand				
	Barusiban	Atosiban	Carbetocin	Oxytocin	[Arg^8]vasopressin
OT	0.8±0.2	52±16	7.1±2.0	0.7±0.2	6.5±0.9
V_2	6300±1200	360±90	5680±1410	310±70	0.6±0.1
E1	6500±1800	1450±230	1170±130	67±5.9	0.6±0.7
E2	8600±2900	1100±290	6860±1400	810±110	1.4±0.2
E3	2900±700	700±140	3850±850	540±90	1.9±0.6
E4	>10000	740±180	6880±1070	670±130	1.3±0.2
E4+	>10000	775±190	8990±1800	940±140	1.6±0.2
E13	>10000	345±50	13±2.6	36±10	1.2±0.2
E123	9880±2150	346±67	56±1.7	38±9.0	5.5±1.3
E1234	8660±2050	390±80	37±7.2	34±7.2	6.7±1.1
TM12	870±130	1050±290	6600±1300	780±130	1.1±0.1
TM12E4+	780±140	1170±220	4470±800	960±120	0.9±0.1

Competition experiments were performed with membranes from COS cells that have been transfected with the indicated receptor constructs (for construct names see Fig. 1B). [^{3}H][Arg^8]vasopressin was used as the radioligand with barusiban, atosiban, carbetocin, oxytocin, and [Arg^8]vasopressin as competitors. The data are K_i values (in nM) and represent means±S.D. from three experiments.

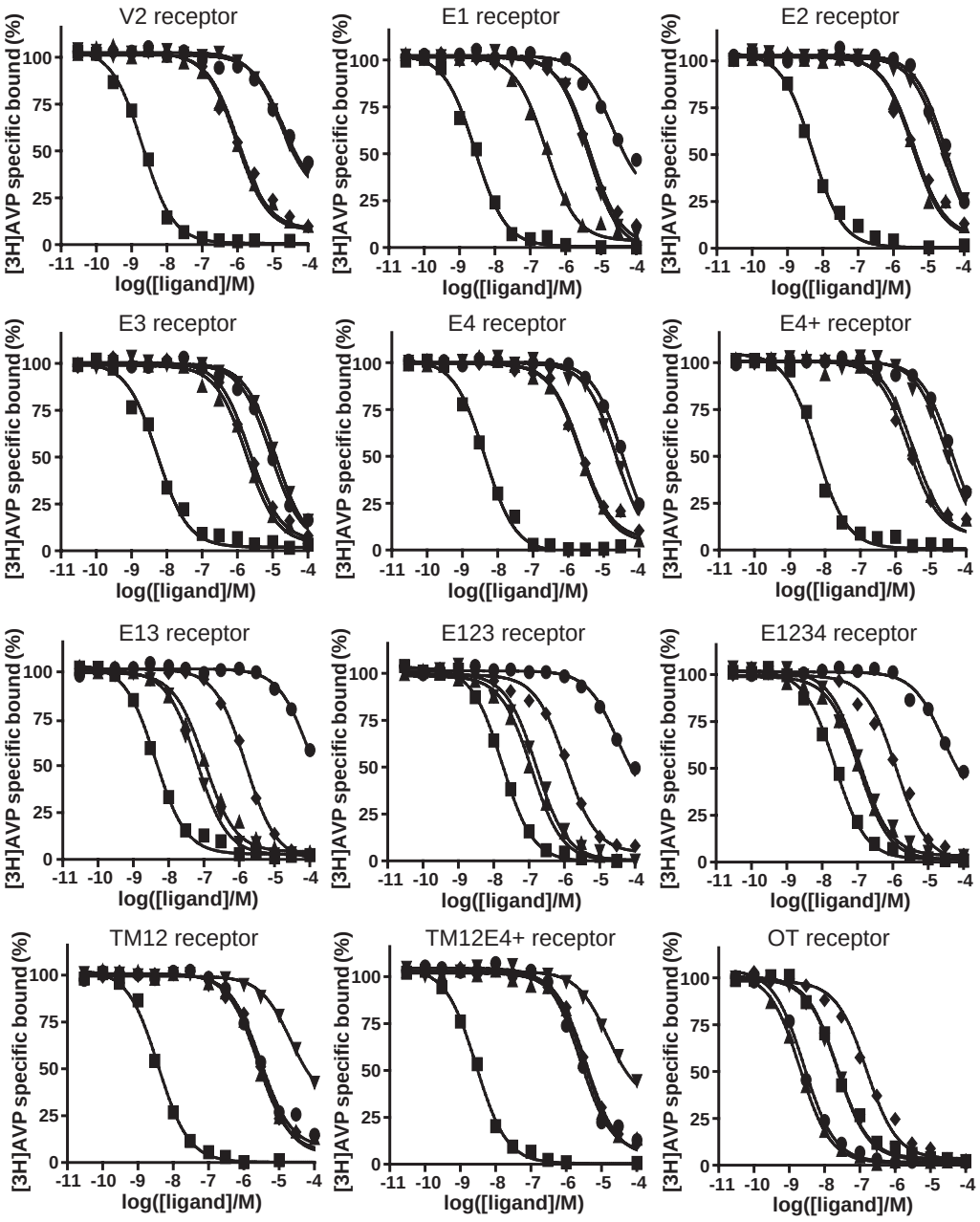

Fig. 2. Competition binding of [^{3}H][Arg^8]vasopressin with the agonists [Arg^8]vasopressin (■), oxytocin (▲) and carbetocin (▼), and the receptor antagonists atosiban (◆), and barusiban (●) as competitors. Data are represented as percentage of bound 3 nM [^{3}H][Arg^8]vasopressin in the absence of unlabeled hormone. Nonspecific binding was determined in the presence of 3 μM [Arg^8]vasopressin. The K_i values have been calculated from the IC_{50} values (obtained by curve fitting of the data) using the Cheng–Prusoff equation: $K_i = IC_{50}/(1+[L]/K_D)$ (for K_i values see Table 1).

genesis, construction of receptor chimeras, photoaffinity labeling, and three-dimensional molecular modeling (Postina et al., 1996; Fanelli et al., 1999; Breton et al., 2001). Here we used chimeric oxytocin/vasopressin V_2 receptor constructs in displacement studies to identify the binding domains of various ligands structurally related to oxytocin. Employment of this strategy allowed us to compare the results with a previous study using the same approach (Postina et al., 1996). Among the ligands tested were barusiban, the most selective oxytocin receptor antagonist available to date, and carbetocin, an agonist of high stability and long duration of action.

The binding profile for oxytocin comprises the extracellular amino-terminus (=E1) and the extracellular loops E2 and E3 of the receptor as previously described (Postina et al., 1996). The contribution of the extracellular amino terminus for oxytocin binding has also been detected by other studies (Hawtin et al., 2001; Wesley et al., 2002). Using site-directed mutagenesis Wesley et al. (2002) identified the amino acid residue R34 within the E1 domain

as essential for oxytocin binding. In all members of the neurohypophysial peptide hormone receptor family, an arginyl is conserved at this locus. This residue might be required for agonist binding throughout this receptor family.

Compared with oxytocin, carbetocin binds to the oxytocin receptor with about 10-fold lower affinity (Engstrom et al., 1998) which is in good agreement with the present results. Here, we found that the binding profile for oxytocin and carbetocin appears to be similar (Fig. 3). This supports the assumption that the N-terminus of the oxytocin receptor is mainly involved in agonist binding and can not select between different agonists (Hawtin et al., 2001; Wesley et al., 2002). As revealed by the crystal structure of rhodopsin, the N-terminus of G-protein coupled receptors could form a compact domain which overlays the extracellular loops (Palczewski et al., 2000; Mirzadegan et al., 2003). Glycosylation sites within the N-terminal part of the oxytocin receptor are most likely not involved in agonist binding. Glycosylation of the oxytocin receptor supports efficient receptor trafficking and

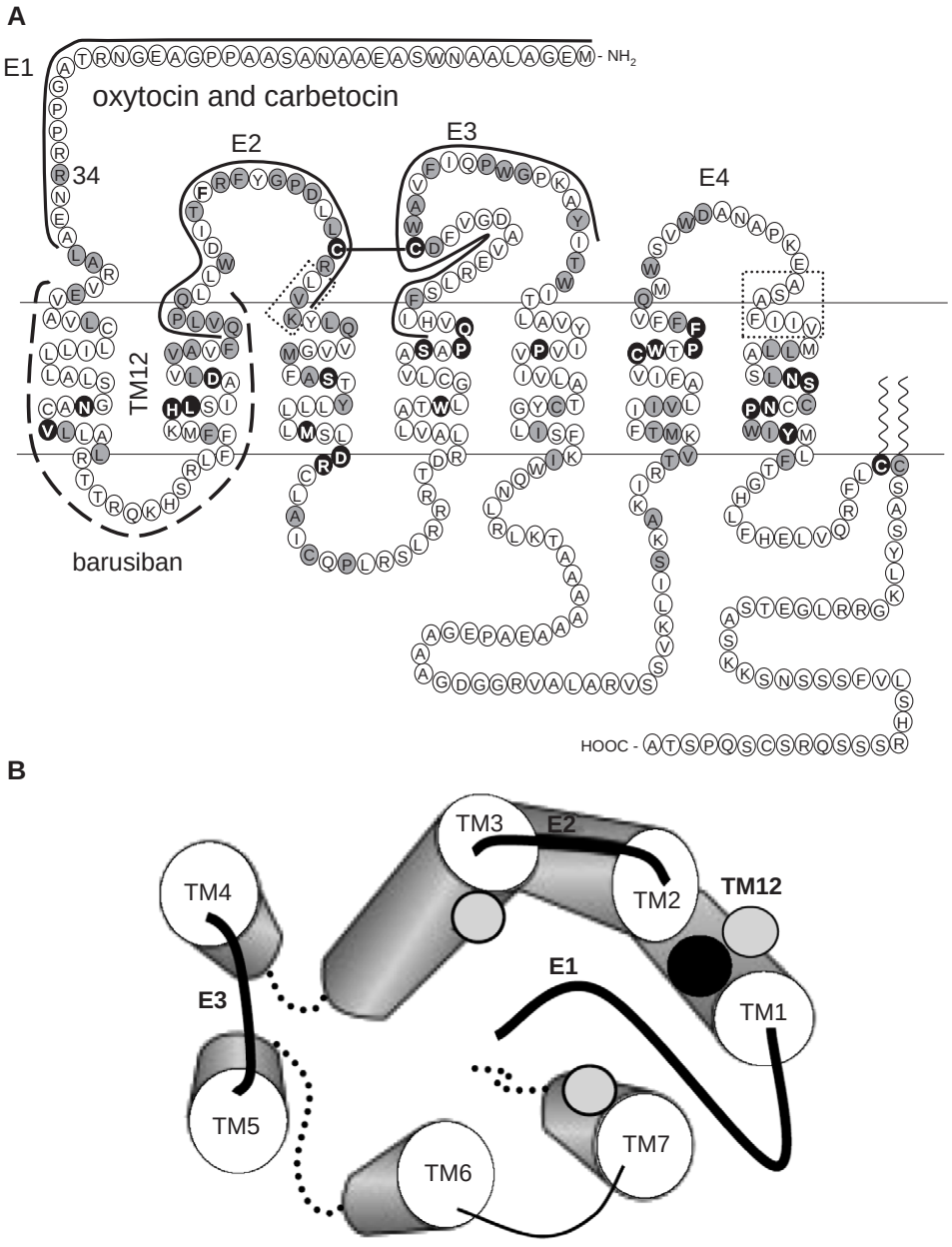

Fig. 3. Models of the oxytocin receptor and identification of binding domains necessary for agonist and antagonist interaction with the oxytocin receptor. A, side view; solid lines, oxytocin and carbetocin binding domains; dashed line, barusiban binding domain; amino acids in boxes, previously defined binding sites for oxytocin receptor antagonists (Postina et al., 1996; Breton et al., 2001). The residues that are conservative within the oxytocin/vasopressin receptor family are outlined in gray, and residues conservative for the whole G-protein coupled receptor family are outlined in black. B, extracellular view onto the oxytocin receptor and localization of receptor agonist and antagonist binding domains (thick lines, oxytocin and carbetocin binding domains; black circle, barusiban binding domains; gray circles, previously defined binding sites for other oxytocin receptor antagonists (Postina et al., 1996; Breton et al., 2001).

increases cell surface expression (Kimura et al., 1997; Wesley et al., 2002).

The binding domains for atosiban could not be evaluated by the present approach. This may be due to the fact that the chimeric approach used here offers only a small 'range of measurements' for atosiban; introduction of oxytocin receptor domains into the vasopressin V_2 receptor could only yield a maximum increase in binding affinity of factor 7 (see K_i values in Table 1) whereas the corresponding factors for oxytocin, carbetocin, and barusiban are much higher, 440, 800, and 7875, respectively.

The binding of barusiban, a highly specific oxytocin receptor antagonist with up to 100-fold more potency than atosiban, was improved when the transmembrane domains 1 and 2 were transferred from the oxytocin receptor to the vasopressin V_2 receptor. Thus, one can conclude that these transmembrane domains directly interact with barusiban. However, the affinity of the receptor construct TM12 to barusiban is still much lower when compared with barusiban's affinity to the oxytocin receptor. This demonstrates that other yet unidentified parts of the oxytocin receptor contribute to the high affinity of barusiban. The transfer of the oxytocin receptor's amino terminus into the vasopressin V_2 receptor (construct E1) did not improve binding of barusiban. Thus, barusiban does not interact with the amino-terminus of the oxytocin receptor. Together with the results from other studies one can now firmly conclude that oxytocin receptor antagonists do not bind to the amino-terminus of the receptor (Elands et al., 1988; Postina et al., 1996; Hawtin et al., 2001; Breton et al., 2001; Wesley et al., 2002).

Transmembrane helices offer a mainly hydrophobic surface for binding contacts. Therefore, one could speculate that hydrophobic parts of barusiban, e.g. the D-Trp at position 2 interact with the hydrophobic transmembrane region. The introduction of certain amino acid sequences at the upper (lipid-extracellular interface) part of transmembrane domain 7 (as present in construct E4+ or TM12E4+) did not improve the binding of barusiban, as it has been observed for $d(CH_2)_5[Tyr\text{-}(Me)^2,Thr^4,Orn^8,Tyr^9]$vasotocin (Postina et al., 1996). The tripeptide sequence 'LVK' at the upper part of transmembrane domain 3 of the oxytocin receptor has been identified as an oxytocin receptor antagonist binding site by a photoaffinity labeling approach (Breton et al., 2001). However, the involvement of the 'LVK' motif for barusiban binding is unlikely, because the transfer of the E2 domain into the vasopressin V_2 receptor did not result in increased affinity for barusiban.

Overall, on the basis of the receptor models in Fig. 3(A and B), one may propose that all ligands structurally related to oxytocin/vasopressin can bind within a central receptor pocket. Due to the anti-clockwise arrangement of the transmembrane helices of the oxytocin receptor (Fig. 3B), amino acid residues that are far apart within the primary sequence are spatially located close together. Different residues located at the central pocket of the receptor can interact with oxytocin receptor agonists or antagonists. The agonists form contact sites with both the 'roof' (i.e. amino terminus) and the extracellular loop domains of the pocket. Obviously, to induce conformational changes of the receptor that lead to signal transduction, the amino terminus (and herein a critical arginyl residue) of the oxytocin receptor has to be contacted by the agonist. In contrast, the oxytocin receptor antagonists prefer contact sites at the bottom of the pocket. In particular, barusiban might gain its increased affinity to the receptor via dipping into the bottom of the pocket between the transmembrane domains 1 and 2. Other approaches such as site-directed mutagenesis or photoaffinity labeling will be necessary to identify the amino acid residues interacting with the different ligands. Ultimately, this should help to establish a precise 3D model of the oxytocin receptor and its binding to oxytocin receptor agonists and antagonists.

Acknowledgements

We thank Christa Wolpert (Mainz) for the excellent technical assistance and Peter McAnulty (Copenhagen) for the review of the manuscript.

References

Akerlund, M., Bossmar, T., Brouard, R., Kostrzewska, A., Laudanski, T., Lemancewicz, A., Serradeil-Le, G.C., Steinwall, M., 1999. Receptor binding of oxytocin and vasopressin antagonists and inhibitory effects on isolated myometrium from preterm and term pregnant women. Br. J. Obstet. Gynaecol. 106, 1047–1053.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 72, 248–254.

Breton, C., Chellil, H., Kabbaj-Benmansour, M., Carnazzi, E., Seyer, R., Phalipou, S., Morin, D., Durroux, T., Zingg, H., Barberis, C., Mouillac, B., 2001. Direct identification of human oxytocin receptor-binding domains using a photoactivatable cyclic peptide antagonist: comparison with the human V1a vasopressin receptor. J. Biol. Chem. 276, 26931–26941.

Elands, J., Barberis, C., Jard, S., Tribollet, E., Dreifuss, J.J., Bankowski, K., Manning, M., Sawyer, W.H., 1988. 125I-labelled d(CH2)5[Tyr (Me)2,Thr4,Tyr-NH2(9)]OVT: a selective oxytocin receptor ligand. Eur. J. Pharmacol. 147, 197–207.

Engstrom, T., Barth, T., Melin, P., Vilhardt, H., 1998. Oxytocin receptor binding and uterotonic activity of carbetocin and its metabolites following enzymatic degradation. Eur. J. Pharmacol. 355, 203–210.

Fanelli, F., Barbier, P., Zanchetta, D., de Benedetti, P.G., Chini, B., 1999. Activation mechanism of human oxytocin receptor: a combined study of experimental and computer-simulated mutagenesis. Mol. Pharmacol. 56, 214–225.

Gimpl, G., Fahrenholz, F., 2001. The oxytocin receptor system: structure, function, and regulation. Physiol. Rev. 81, 629–683.

Gorbulev, V., Buchner, H., Akhundova, A., Fahrenholz, F., 1993. Molecular cloning and functional characterization of V2 [8-lysine] vasopressin and oxytocin receptors from a pig kidney cell line. Eur. J. Biochem. 215, 1–7.

Hawtin, S.R., Howard, H.C., Wheatley, M., 2001. Identification of an extracellular segment of the oxytocin receptor providing agonist-specific binding epitopes. Biochem. J. 354, 465–472.

Kimura, T., Makino, Y., Bathgate, R., Ivell, R., Nobunaga, T., Kubota, Y., Kumazawa, I., Saji, F., Murata, Y., Nishihara, T., Hashimoto, M., Kinoshita, M., 1997. The role of N-terminal glycosylation in the human oxytocin receptor. Mol. Hum. Reprod. 3, 957–963.

Manning, M., Cheng, L.L., Klis, W.A., Stoev, S., Przybylski, J., Bankowski, K., Sawyer, W.H., Barberis, C., Chan, W.Y., 1995. Advances in the design of selective antagonists, potential tocolytics, and radioiodinated ligands for oxytocin receptors. Adv. Exp. Med. Biol. 395, 559–583.

Mirzadegan, T., Benko, G., Filipek, S., Palczewski, K., 2003. Sequence analyses of G-protein-coupled receptors: similarities to rhodopsin. Biochemistry 42, 2759–2767.

Nilsson, L., Reinheimer, T., Steinwall, M., Akerlund, M., 2003. FE 200 440: a selective oxytocin antagonist on the term-pregnant human uterus. BJOG 110, 1025–1028.

Palczewski, K., Kumasaka, T., Hori, T., Behnke, C.A., Motoshima, H., Fox, B.A., Le, T.I., Teller, D.C., Okada, T., Stenkamp, R.E., Yamamoto, M., Miyano, M., 2000. Crystal structure of rhodopsin: a G protein-coupled receptor. Science 289, 739–745.

Pierzynski, P., Lemancewicz, A., Reinheimer, T., Akerlund, M., Laudanski, T., 2004. Inhibitory effect of barusiban and atosiban on oxytocin-induced contractions of myometrium from preterm and term pregnant women. J. Soc. Gynecol. Investig. 11, 384–387.

Postina, R., Kojro, E., Fahrenholz, F., 1996. Separate agonist and peptide antagonist binding sites of the oxytocin receptor defined by their transfer into the V2 vasopressin receptor. J. Biol. Chem. 271, 31593–31601.

Ufer, E., Postina, R., Gorbulev, V., Fahrenholz, F., 1995. An extracellular residue determines the agonist specificity of V2 vasopressin receptors. FEBS Lett. 362, 19–23.

Wesley, V.J., Hawtin, S.R., Howard, H.C., Wheatley, M., 2002. Agonist-specific, high-affinity binding epitopes are contributed by an arginine in the N-terminus of the human oxytocin receptor. Biochemistry 41, 5086–5092.