ELSEVIER

European Journal of Pharmacology 347 (1998) 293–299

ejp

Are high-affinity progesterone binding site(s) from porcine liver microsomes members of the σ receptor family?

Christiane Meyer [a], Kerstin Schmieding [a], Elisabeth Falkenstein [b], Martin Wehling [a,b,*]

[a] *Division of Clinical Pharmacology, Medizinische Klinik, Klinikum Innenstadt, University of Munich, D-80336 Munich, Germany*
[b] *Institut für Klinische Pharmakologie, Fakultät für Klinische Medizin Mannheim, Ruprecht-Karls-Universität Heidelberg, Klinikum Mannheim, Theodor-Kutzer-Ufer, D-68167 Mannheim, Germany*

Received 14 August 1997; revised 28 January 1998; accepted 3 February 1998

Abstract

Membrane progesterone binding sites have been purified recently from pig liver. Since progesterone is considered as an endogenous sigma (σ) receptor ligand, these sites were characterized pharmacologically by ligands selective for σ receptor and dopamine receptor binding sites, and by other drugs from distinct pharmacological classes. Binding studies using the radioligand [^{3}H]progesterone were done in crude membrane preparations and solubilized fractions to determine half-maximal inhibitory concentration (IC_{50}) values, from which inhibitory constants (K_i values) were calculated. Radioligand binding was inhibited by the σ receptor ligands haloperidol, carbetapentane citrate, 1,3-Di(2-tolyl)guanidine (DTG), *R*(−)-*N*-(3-phenyl-1-propyl)-1-phenyl-2 aminopropane HCl (*R*(−)-PPAAP HCl), or σ receptor antagonists like (+)-3-(3-hydroxyphenyl)-*N*-propylpiperidine HCl (*R*(+)-PPP HCl) and *cis*-9-[3-(3,5-dimethyl-1-piperazinyl)propyl]-9*H*-carbazole dihydrochloride (rimcazole 2HCl). The hierarchy of inhibitory action was not fully compatible with either σ receptor class I (moderate affinity of pentazocine, diphenylhydantoin (phenytoin) insensitivity) or II sites (high affinity of carbetapentane). The data thus suggest that progesterone binding sites in porcine liver membranes are related to the σ receptor binding site superfamily, but may represent a particular species with progesterone specificity. © 1998 Elsevier Science B.V.

Keywords: Progesterone; σ-receptor; Membrane receptor; Microsome, pig, liver

1. Introduction

Sigma (σ) binding sites have originally been described as a class of opiate receptor sites (Martin et al., 1976). Subsequent investigations, however, lead to a revision of this concept showing that σ receptor sites are distinct from any known drug or neurotransmitter receptor (Sonders et al., 1988). As the function of these sites is yet unknown, the molecular characterization of σ receptor sites and their possible structural relationship to other known membrane receptors is of increasing interest at present. Photoaffinity labeling of σ_1 receptor sites tagged different polypeptides with molecular masses ranging from 25–29 kDa (Su, 1991). Schuster et al. (1994) described the purification of a 28-kDa cyclophilin-like component of the rat liver σ receptor by affinity chromatography using haloperidol as affinity ligand. Moebius et al. (1993, 1994) and Hanner et al. (1995) identified an phenylalkylamine Ca^{2+} channel antagonist binding protein (27 kDa) from guinea pig liver which showed high affinity to σ receptor ligands. Recently, Hanner et al. (1996) reported the purification and molecular cloning of a 29.7-kDa polypeptide from guinea pig liver showing essential properties of a σ receptor site type I. All binding sites were found to recognize selective σ receptor ligands with high affinity, but surprisingly, no structural relationship of their primary structure was observed.

Progesterone is an established ligand for σ receptor binding sites and considered as a potential endogenous ligand of these sites (Su, 1991). Recently, we have purified and cloned a high-affinity membrane progesterone binding protein (28 kDa) (Meyer et al., 1996; Falkenstein et al., 1996) which may be involved in rapid, non-genomic effects of steroids (Wehling, 1995, 1997), although functional expression has still to be achieved.

In the present study, the binding profile of this progesterone membrane binding site is characterized with particular reference to σ receptor ligands and neurotransmitter receptor ligands.

* Corresponding author at address b. Tel.: +49-621-383-4058; fax: +49-621-383-2024; e-mail: wehling@medinn.med.uni-muenchen.de

2. Materials and methods

2.1. Chemicals and reagents

[1,2,6,7-^{3}H(N)]progesterone (spec. act. 96 Ci mmol^{-1} = 3.564 TBq mmol^{-1}) and [1,3-^{3}H](+)-pentazocine (spec. act. 35 Ci mmol^{-1} = 1.3 TBq mmol^{-1}) were obtained from NEN (Bad Homburg, Germany). Unlabeled progesterone was purchased from Sigma (Munich, Germany), other ligands (e.g., σ receptor ligands) were from Calbiochem (Bad Soden, Germany), Biotrend Chemikalien (Cologne, Germany) and ICN Biomedicals (Eschwege, Germany). All other chemicals were of reagent grade and obtained from Merck (Darmstadt, Germany), Boehringer (Mannheim, Germany), Sigma (Munich, Germany), or Serva (Heidelberg, Germany). The phenylalkylamines (+)-emopamil and (−)-emopamil were kindly provided by Knoll (Ludwigshafen, Germany).

2.2. Membrane preparation and solubilization of progesterone-binding site(s)

Crude microsomal membranes were prepared by differential centrifugation from porcine tissues as described in detail elsewhere (Meyer et al., 1996). Fresh tissue was obtained from the slaughterhouse. Microsomal 100 000 × g pellets were washed two times in homogenization buffer (buffer A; 50 mM Tris/HCl, pH 7.5 containing 0.25 M sucrose, 0.2 mM phenylmethylsulfonyl fluoride and 0.5 μg/ml leupeptin); aliquots (10–25 mg protein ml^{-1}) were subsequently frozen in liquid nitrogen and stored at −80°C until used. Microsomal membrane proteins from porcine liver were solubilized at a detergent:protein ratio of 4:1 (w/w) in buffer B (50 mM Tris/Mes, pH 6.0, containing 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM KCl) with 20 mM 3-[(3-cholamidoprpyl) dimethylammonio]-1-propansulfonic acid (Chaps) (Boehringer, Mannheim, Germany) in the presence of 0.2 mM phenylmethylsulfonyl fluoride and 200 mM KCl. In all experiments, either crude microsomal membranes or solubilized proteins were used as indicated.

2.3. Progesterone-binding assay

Binding assays were performed according to Meyer et al. (1996). Briefly, microsomal or solubilized proteins (typically 200 μg protein) were incubated in buffer B (volume 0.5 ml) in the presence of 20 nM [^{3}H]progesterone. Non-specific binding was determined in all experiments in the presence of a 1000-fold excess of unlabeled progesterone and amounted to 20% (Fig. 1A). This assay was used as a control experiment for all inhibition experiments. In competition experiments, half-maximal inhibitory concentrations (IC_{50}) were obtained by nonlinear fitting of inhibition curves (Sigma plot®, Version 1.02, Jandel). We calculated inhibitory constants (K_i) from IC_{50} as described by Cheng and Prusoff (1973). All compounds were tested at concentrations ranging from 0–100 μM in the presence of 20 nM [^{3}H]progesterone. Light-sensitive compounds were tested in the dark room. In all figures, percentages of total binding are given. Mean relative standard deviation (S.D.) of K_i was 16 ± 12% (mean ± S.D.). In some experiments, 20 nM [^{3}H](+)-pentazocine instead of [^{3}H]progesterone was used as radioli-

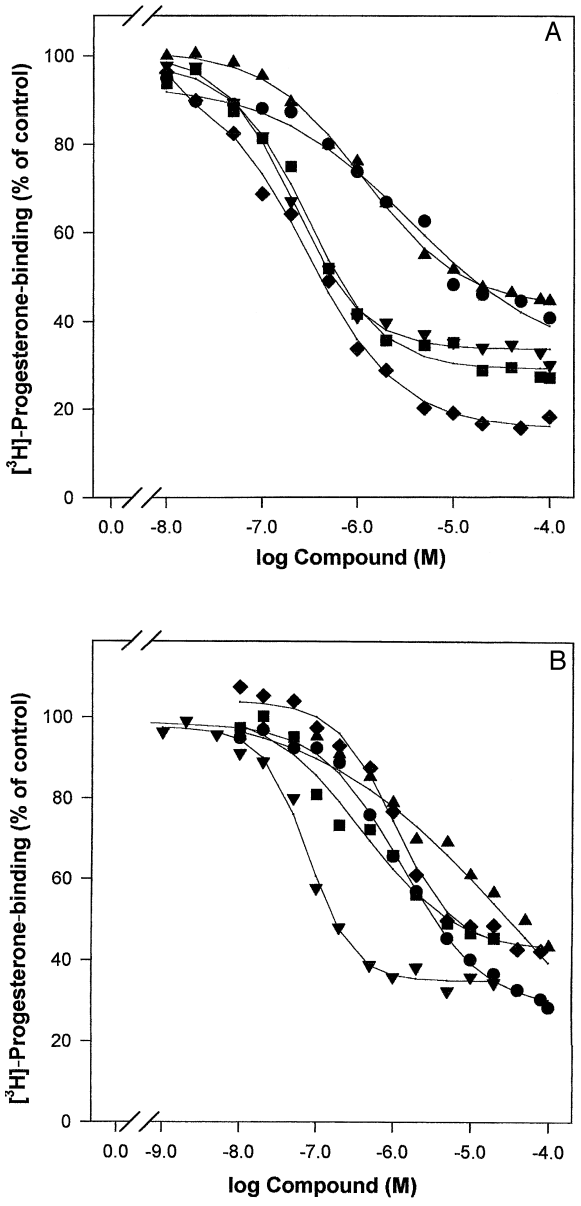

Fig. 1. Dose–response competition curves of σ_1 receptor discriminating ligands (A) and nondiscriminating ligands (B) on porcine liver microsomal fractions using [^{3}H]progesterone as radioligand (20 nM). Data are from representative individual experiments. For comparison, the displacement curve for unlabeled progesterone was added in (A). Values given represent percentages of total binding compared with control. (A) (+)-SKF10,047 (●); carbetapentane citrate (■); (±)-pentazocine (▲); R(−)-PPAP (▼); progesterone (◆). (B) Rimcazole (●); DTG (■); (−)-SKF10,047 (▲); haloperidol (▼); (+)-3-PPP (◆).

gands, and non-specific binding was determined in the presence of 20 μM (±)-pentazocine (base).

Diphenylhydantion (phenytoin) sensitivity was tested by comparing binding values in the presence or absence of these compounds. Increases of binding by > 20% were considered to indicate 'sensitivity'.

The data are presented as means ± S.D. of at least two experiments run in duplicate. For statistical comparison, Student's *t*-test was used. *P*-values < 0.01 were considered statistically significant.

2.4. Protein determination

Protein was determined by the Bio-Rad protein assay kit with bovine serum albumin as reference standard (Bradford, 1976). If solubilized protein concentrations were measured, the standard curve was established in the presence of detergent.

3. Results

The affinities of different compounds were tested with regard to [^{3}H]progesterone binding to crude microsomal membrane fractions and solubilized membranes from porcine liver and expressed by their K_i values (μM). As this involves many drugs, the rationale of their use with regard to the pharmacological characterization of progesterone membrane binding sites is mentioned here rather than in Section 4. It should be also noted, that the tracer concentration (20 nM [^{3}H]progesterone) was chosen to predominantly label high-affinity sites (K_{d1} 11 nM), rather than lower affinity sites (K_{d2} 286 nM) (Meyer et al., 1996). An even lower concentration appeared not to be feasible as resulting cpm were too low for accurate determination. This assumption is supported by experiments in solubilized membrane fractions, which for that reason, have always been carried out in addition to those in microsomes. In these, the low-affinity site is lost (Meyer et al., 1996), and the high-affinity site is the only one present. As the results are similar in both preparations, especially with regard to the hierarchy of affinities, a more extensive, two-site model analysis appeared non-essential for the interpretation of results and, therefore, was not done. The higher K_{d1} in solubilized fractions (69 nM) apparently is the reason for the higher K_i values in solubilized fractions compared with those than in microsomal fractions.

3.1. σ Receptor ligands

All σ receptor ligands tested showed high-to-low affinities for progesterone binding sites (Table 1). Non-discriminatory (non-selective) σ receptor ligands (Quirion et al., 1992) such as haloperidol, (+)-3-(3-hydroxyphenyl)-*N*-propylpiperidine HCl (*R*(+)-3-PPP HCl) (Koe et al., 1989), 1,3-Di(2-tolyl)guanidine (DTG), and (−)-*N*-allylnormetazocine ((−)-SKF10,047) were found to be active in competition experiments in a hierarchical order

Table 1
Inhibition of [^{3}H]progesterone binding by σ receptor ligands in microsomal membrane preparations and solubilized fractions of porcine liver

Compound	Selectivity sigma receptor	K_i (μM)	
		Microsomal membranes	Solubilized membranes
Discriminant receptor ligands			
R(−)-PPAP HCl	σ_1-agonist	0.07	0.08
Carbetapentane citrate	σ_1-agonist	0.09	0.42
(+)-SKF10,047	σ_1-agonist	1.2	–
Nondiscriminant receptor ligands			
Haloperidol	σ-agonist	0.02	0.07
R(+)-3-PPP HCl	σ-antagonist	0.29	0.86
DTG	σ-agonist	0.31	1.87
(−)-SKF10,047	σ-agonist	12.7	12.63
Further σ receptor ligands			
Haloperidol (reduced)	σ-agonist	0.08	0.64
Ifenprodil tartrate	σ-agonist	0.09	0.25
cis-1*S*,2*R*-U-50488	σ-agonist	0.44	0.82
Rimcazole-2HCl	σ-antagonist	0.51	2.03
S(−)-3-PPP HCl	σ-antagonist	0.61	2.71
(±)-Pentazocine (base)	σ-agonist	1.13	6.35
(±)-SKF10,047	σ-agonist	4.61	6.29
Enhancer			
Phenytoin	Enhancer	Inactive	Inactive

Microsomal or solubilized fractions were incubated with 20 nM [^{3}H]progesterone in the presence or absence of different concentrations of the tested drug. The affinities were expressed as K_i values.

compatible with the one proposed for the general identification of σ receptor sites (Fig. 1B).

Additionally, we observed moderate-to-high affinities of selective σ receptor ligands. These discriminant ligands are used to classify two subtypes of σ receptor binding sites (Quirion et al., 1992). The benzomorphane derivative σ receptor agonist (+)-SKF10,047 inhibited progesterone binding stereoselectively, the (+)-isomer was about 10 × more active than (−)-SKF10,047 (Table 1). This criteria is typical for σ_1 receptor binding sites (Quirion et al., 1992). Moreover, as shown in Fig. 1A, other σ receptor ligands tested had high affinities for progesterone binding: carbetapentane citrate ($K_i = 90$ nM), a ligand which is used as a tool to discriminate between σ_1 receptor and σ_2 receptor sites (Quirion et al., 1992) had a high affinity to progesterone binding sites as well as *R*(−)-*N*-(3-phenyl-1-propyl)-1-phenyl-2 aminopropane HCl (*R*(−)-PPAP HCl) ($K_i = 70$ nM) (Glennon et al., 1990), which were compatible with the assumption that progesterone binding site(s) may belong to the σ receptor binding sites superfamily, subtype 1. However, the racemate of pentazocine (base) (Brodgen et al., 1973) displaced the progesterone binding with moderate affinity of $K_i = 1.13$ μM (Table 1). (+)-pentazocine as a high affinity selective σ_1 receptor ligand was available as radioligand only. The racemate should be only 30% less active than (+)-pentazocine (Cagnotto et al., 1994). Given this, (+)-pentazocine would be expected to be still only moderately active here—a feature that is incompatible with the classification of progesterone binding sites as σ_1 receptor sites, which should expose high affinity for (+)-pentazocine. In addition, phenytoin was inactive as enhancer of progesterone binding (Table 1); this is also not typical for σ_1 receptor sites.

3.2. Dopamine receptor ligands

As summarized for antidopaminergic compounds in Table 2, the antipsychotic dopamine D_2 receptor antagonist, haloperidol, dopamine D_1 receptor antagonists fluphenazine and proadifen, dopamine receptor antagonist and less active enantiomer, (−)-butaclamol, the dopamine D_2 receptor agonist, *R*(+)-3-PPP HCl, and the dopamine uptake blocker, buspirone HCl, showed high affinity ($K_i < 0.35$ μM) for the progesterone binding sites while the dopamine receptor antagonists chlorpromazine HCl, pimozide and clozapine had moderate or low affinities. Remarkably, the endogenous dopamine receptor ligand, dopamine HCl, did not compete with progesterone binding. These results indicate that progesterone binding sites may bind dopamine receptor ligands, especially dopamine D_2 receptor ligands, but the hierarchy does not match with clinical activity. For example, the dopamine D_2 receptor agonist, *S*(−)-lisuride, and the dopamine receptor antagonist, (−)-sulpiride, do not block progesterone binding at

Table 2
Inhibitory potencies of various agonists and antagonists of dopamine receptors in porcine liver tissue on the binding of [^{3}H]progesterone in microsomal membrane preparations and solubilized fractions

Compound	Selectivity dopamine receptor	K_i (μM)	
		Microsomal membranes	Solubilized membranes
Haloperidol	D_2/D_1-antagonist	0.02	0.07
Fluphenazine	D_1/D_2-antagonist	0.03	0.14
Buspirone HCl	Dopamine uptake inhibitor	0.11	0.31
(−)-Butaclamol HCl	D-antagonist	0.11	0.14
Trifluoperazine	D_1/D_2-antagonist	0.12	0.4
Proadifen (SKF 525A)	D_1/D_2-antagonist	0.14	0.17
R(+)-3-PPP HCl	D_2-agonist	0.29	0.86
Spiperone HCl	D_2-antagonist	0.31	4.04
S(−)-Raclopride L-tartrate	D_2-antagonist	0.33	1.38
Chlorpromazine HCl	D-antagonist	0.38	1.06
S(−)-3-PPP HCl	D_2-agonist	0.61	2.73
Pimozide	D_2-antagonist	0.73	1.3
(+)-Butaclamol HCl	D_2/D_1-antagonist	2.21	1.44
Clozapine	$D_4 > D_3 > D_2$ antagonist	17.31	Inactive
R(+)-SCH-23390 HCl	D_1-agonist	Inactive	Inactive
S(−)-SCH-23388 HCl	D_1-agonist	Inactive	Inactive
R(+)-6-Bromo-APR HBr[a]	D_1-agonist	Inactive	Inactive
R(−)-SCH-12679 maleate	D_1-antagonist	Inactive	Inactive
S(−)-Lisuride	D_2-agonist	Inactive	Inactive
(−)-Sulpiride	D_2-antagonist	Inactive	Inactive
S(+)-PD128,907 HCl	D_3-antagonist	Inactive	Inactive
Dopamine HCl	Endogenous ligand	Inactive	Inactive

Affinities were expressed as K_i values.
Inactive: not active up to 10^{-4} M.
[a] *R*(+)-6-bromo-APR HBr: *R*(+)-6-bromo-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrobromide.

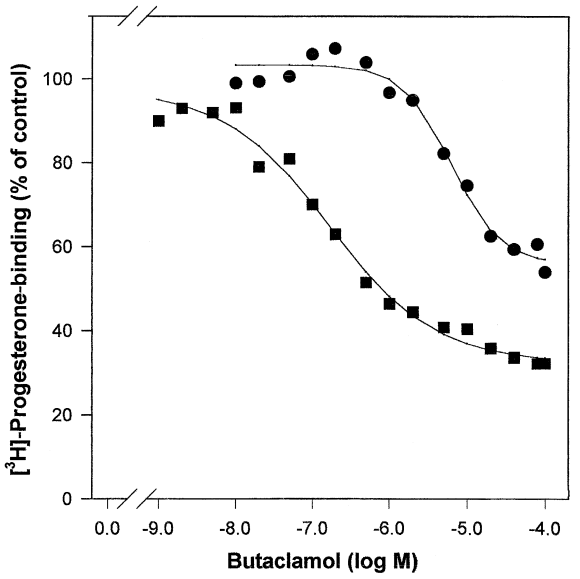

Fig. 2. Stereoselectivity of [^{3}H]progesterone binding site(s) in porcine liver membrane fractions for (−)-butaclamol (■) and (+)-butaclamol (●). Representative displacement curves are shown. Values given represent percentages of total binding compared with control.

concentrations up to 100 μM. Moreover, (−)-butaclamol HCl is known to be the enantiomer less active on dopamine receptors (Su, 1993), but an opposite stereoselectivity was observed in the case of progesterone binding site(s) (Fig. 2): (−)-butaclamol HCl was found to be more active than (+)-butaclamol HCl. This enantioselectivity for butaclamol HCl was described to be typical for σ receptor sites (Weber et al., 1986).

3.3. Ligands from different pharmacological groups

Compounds from various further pharmacological groups were tested with regard to their affinities to progesterone binding sites (data not shown). High-affinity compounds not mentioned so far include monamine oxidase uptake inhibitors type A (clorgyline), Ca^{2+} channel antagonists of the phenylalkyamine type ((+)-/(−)-emopamil). Ca^{2+} channel blockers (gadolinium-III-chloride, lanthanum chloride), cytochrome P_{450} inhibitors (methyrapone, cimetidine), α-adrenoceptor antagonists (yohimbine, phentolamine), Na^+/H^+ antiporter inhibitor (amiloride), K^+ channel blocker (4-aminopyridine), opiate receptor antagonist (naloxone HCl), and serotonin were inactive ($K_i > 35$ μM). The β-adrenoceptor antagonist, (±)-propranolol HCl and its enantiomers showed moderate affinities to the progesterone binding site(s).

It should be noted that maximum displacement varied for different inhibitors of binding: pentazocine at high concentrations reduced binding to only $41 \pm 4\%$ ($n = 4$, $P < 0.01$ vs. progesterone), $R(-)$-PPAP HCl to $31 \pm 3\%$ ($n = 4$, $P < 0.01$ vs. progesterone) and carbetapentane citrate to $32 \pm 4\%$ ($n = 4$, $p < 0.01$ vs. progesterone) compared with $15 \pm 1\%$ ($n = 5$) for progesterone (Fig. 1A). In turn, with [^{3}H]pentazocine as radioligand, maximum effect of progesterone on binding was $56 \pm 4\%$ ($n = 4$, $P < 0.01$) of that by unlabeled pentazocine (not shown) possibly pointing to the involvement of σ and additional, distinct sites.

4. Discussion

The main findings of the present study are as follows: (1) progesterone binding sites in pig liver membranes specifically bind σ receptor ligands; (2) the hierarchy of binding is typical for σ receptor sites, in general; however, (3) the hierarchy of binding and other properties (phenytoin sensitivity) are not typical in particular for either σ_1 or σ_2 receptor sites.

The high-affinity progesterone binding site(s) investigated here have been studied with regard to steroid binding properties (Meyer et al., 1996). A selectivity for progesterone over all other steroids tested was determined, leading to the term ‘progesterone binding sites’. Related steroids were recognized in the following order of relative affinities: progesterone > corticosterone ≥ testosterone > cortisol > promegestone > fludrocortisone = β-estradiol = canrenone = dexamethasone = aldosterone (Meyer et al., 1996). Scatchard analysis of saturation experiments revealed the presence of high-affinity and low-affinity sites in microsomes with apparent dissociation constants of 11 ± 0.54 nM and 286 ± 40 nM, respectively. The capacities were 3.4 ± 0.1 pmol mg protein^{-1} for the high-affinity and 44 ± 4.4 pmol mg protein^{-1} for the low-affinity binding sites. Furthermore, radioligand binding studies on microsomal and solubilized protein indicated that only the high-affinity sites were solubilized, which shows biochemical characteristics similar to those of microsomal binding proteins (Meyer et al., 1996). The loss of the low-affinity binding site may be due to the cross-reactivity of progesterone exposing affinities for other steroid membrane binding sites such as microsomal aldosterone binding sites, which have also been identified in porcine liver (Meyer et al., 1995). An additional explanation could be the removal of steroid binding membrane lipids by the detergent (Meyer et al., 1996).

As progesterone binding to membrane sites is a typical feature of σ sites, the rationale of the present study was to identify further pharmacological properties of progesterone binding sites relevant to this interrelation.

Various drugs and chemicals with several different biological/pharmacological profiles were tested for their ability to displace radioligand binding to microsomal membranes and solubilized fractions from porcine liver. The choice of compounds was guided by earlier reports on the characterization of σ receptor sites with particular reference to the classification proposal by Quirion et al. (1992).

Taking results on discriminant and non-discriminant σ receptor ligands together, the picture is fully compatible with the assumption that these progesterone binding sites are σ receptor sites, and the pharmacological profile differs essentially from those of known dopaminergic, serotonergic, adrenergic, phenycyclidine (PCP) (Itzhak, 1988), and opioid receptors (Su, 1993), as well as cytochrome P_{450} and monoamine oxidase enzymes. With regard to the σ receptor subclassification, the low affinity of pentazocine and insensitivity to phenytoin as promoter of binding are not in line with properties typical for σ_1 receptor sites. Reasons for this could be the radioligand employed and its concentration. 20 nM [^{3}H]progesterone is not expected to label a large proportion of those binding sites termed σ_1 receptor sites, which are labeled by [^{3}H]pentazocine at a 10–100-fold higher affinity than by progesterone (Cagnotto et al., 1994; Hanner et al., 1996). Estimated K_d for progesterone binding to σ_1 receptor sites is >250 nM (Hanner et al., 1996), thus essentially excluding [^{3}H]progesterone from binding to σ_1 receptor sites at the concentrations employed. This, therefore, may explain why pentazocine has only moderate affinity. In turn, it has been shown that progesterone and haloperidol bind to rat liver membranes with displacement by various ligands exposing a similar pharmacological profile (Yamada et al., 1994). Haloperidol and DTG are high affinity ligands for both types of σ receptor sites. Therefore, progesterone may be considered as a suitable non-discriminant ligand for σ receptor sites, and this even more so, as for the detection of σ_2 receptor sites by haloperidol and DTG, saturating concentrations of (+)-pentazocine or other σ_1 receptor specific ligands must be present. As progesterone does not essentially occupy σ_1 receptor sites at the concentration employed, addition of σ_1 receptor specific ligands is not necessary with progesterone, which may even be considered σ_2 receptor-specific. However, though low affinity for pentazocine and insensitivity to phenytoin support classification as σ_2 receptor sites, other properties do not: σ_1 receptor specific ligands $R(-)$-PPAP HCl (Glennon et al., 1990), carbetapentane citrate and (+)-SKF10,047, but not (−)-SKF10,047 show high-to-moderate affinities being characteristic for σ_1 receptor sites (Quirion et al., 1992). How to reconcile these apparent discrepancies? Only recently, the first σ_1 receptor site has been cloned (Hanner et al., 1996), and functionally expressed. It is suggested that phenylalkylamine Ca^{2+} channel antagonist binding to this protein may be pharmacologically relevant (Hanner et al., 1995). In an earlier paper, the same group identified a different phenylalkylamine-binding polypeptide as possible σ_1 receptor binding site by photoaffinity labeling and ligand-directed antibodies (Moebius et al., 1993). This protein was thought to have four membrane spanning regions rather than one as shown for the first one. By all means, the recently cloned 28-kDa protein exposes the pharmacological properties typical of σ_1 receptor sites, and it was isolated by [^{3}H]pentazocine-labeling. In contrast, the primary structure of the progesterone binding, 28-kDa protein isolated and cloned in our laboratory does not share any structural homology with former protein. As shown by antibody labeling, it is widespread within and between species (Meyer, unpublished data), properties typical for σ receptor sites (Walker et al., 1990) which are expressed in the central nervous system, endocrine, immune and certain peripheral tissues such as liver.

Photoaffinity labelling of σ receptor binding sites showed a molecular mass of 24–29 kDa (Su, 1991). This was done in guinea pig, rat and bovine brain and guinea pig liver preparations using the highly selective σ receptor ligand [^{3}H]azido-DTG (Schuster et al., 1994, 1995; Kavanaugh et al., 1988) and in rat liver, rat brain, and human placenta (26 kDa) with the selective σ receptor ligand [^{125}I]azidococaine (Kahoun and Ruoho, 1992). These results are in line with a molecular mass of 28 kDa determined for the subunit(s) of the progesterone binding protein described by us. N-terminal amino acid sequencing showed an identical sequence at 56 kDa pointing to a possible dimerization of 28-kDa polypeptides. The molecular mass of σ receptor binding site complexes was estimated at 150–450 kDa, and depended on the detergent used (Walker et al., 1990; Kavanaugh et al., 1988; Schuster et al., 1995). We determined an apparent complex size of about 200 kDa by gel permeation using CHAPS as detergent (Meyer et al., 1998). As a hypothesis that would have to be substantiated by the functional expression of the cloned progesterone binding protein, these sites could represent a third species of σ receptor binding sites with progesterone specificity. In conclusion, liver microsome progesterone binding sites share major similarities with σ receptor sites, although neither σ_1 receptor nor σ_2 receptor subclassification can be satisfactorily applied. Further molecular analysis is necessary to define essential properties of these progesterone σ receptor sites with regard to pharmacology and pathophysiology.

Acknowledgements

We thank Martina Krautkrämer and Ulrich Katzner for expert technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft (Sc 4/9-4, Sc 4/9-5, We 1184/6-1).

References

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

Brodgen, R.N., Speight, T.M., Avery, G.S., 1973. Pentazocine: a review of its pharmacological properties, therapeutics efficacy and dependence liability. Drug 5, 6–91.

Cagnotto, A., Bastone, A., Mennini, T., 1994. [^{3}H](+)-Pentazocine binding to rat brain σ_1 receptors. Eur. J. Pharmacol. 266, 131–138.

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

Falkenstein, E., Meyer, C., Eisen, C., Scriba, P.C., Wehling, M., 1996. Full-length cDNA sequence of a progesterone membrane binding protein from porcine vascular smooth muscle cells. Biochem. Biophys. Res. Commun. 229, 86–89.

Glennon, R.A., Battaglia, G., Smith, J.D., 1990. (−)PPAP: A new selective ligand for sigma binding sites. Pharmacol. Biochem. Behav. 37, 557–559.

Hanner, M., Moebius, F.F., Weber, F., Grabner, M., Striessnig, J., Glossmann, H., 1995. Phenylalkylamine Ca^{2+} antagonist binding protein. J. Biol. Chem. 270, 7551–7557.

Hanner, M., Moebius, F.F., Flandorfer, A., Knaus, H.-G., Striessnig, J., Kempner, E., Glossmann, H., 1996. Purification, molecular cloning, and expression of the mammalian sigma$_1$-binding site. Proc. Natl. Acad. Sci. USA 93, 8072–8077.

Itzhak, Y., 1988. Pharmacological specificity of some psychotomimetic and antipsychotic agents for the σ and PCP binding sites. Life Sci. 42, 745–752.

Kahoun, J.R., Ruoho, A.E., 1992. (^{125}I)Iodoazidococaine, a photoaffinity label for the haloperidol-sensitive sigma receptor. Proc. Natl. Acad. Sci. USA 89, 1393–1397.

Kavanaugh, M.P., Tester, B.C., Scherz, M.W., Keana, J.F.W., Weber, E., 1988. Identification of the binding subunit of the σ-type opiate receptor by photoaffinity labeling with 1-(4-azido-2-methyl[6-^{3}H]phenyl)-3-(2-methyl[4,6-^{3}H]phenyl)guanidine. Proc. Natl. Acad. Sci. USA 85, 2844–2848.

Koe, B.K., Burkhart, C.A., Lebel, L.A., 1989. (+)-[^{3}H]3-(3-Hydroxyphenyl)-*N*-(1-propyl)-piperidine binding to sigma receptors in mouse brain in vivo. Eur. J. Pharmacol. 161, 263–266.

Martin, W.R., Eades, C.G., Thompson, J.A., Huppler, R.E., Gilbert, P.E., 1976. The effects of morphine- and nalorphine-like drugs in the non-dependent and morphine-dependent chronic spinal dog. J. Pharmacol. Exp. Ther. 197, 517–532.

Meyer, C., Christ, M., Wehling, M., 1995. Characterization and solubilization of novel aldosterone-binding-proteins in porcine liver microsomes. Eur. J. Biochem. 229, 736–740.

Meyer, C., Schmid, R., Scriba, P.C., Wehling, M., 1996. Purification and partial sequencing of a putative progesterone-receptor from porcine liver membranes. Eur. J. Biochem. 239, 726–731.

Meyer, C., Schmid, R., Schmieding, K., Falkenstein, E., Wehling, M., 1998. Characterization of high affinity progesterone-membrane binding proteins by an anti-peptide antiserum. Steroids, in press.

Moebius, F.F., Burrows, G.G., Hanner, M., Schmid, E., Striessnig, J., Glossmann, H., 1993. Identification of a 27-kDa high affinity phenylalkylamine-binding polypeptide as the σ_1 binding site by photoaffinity labeling and ligand-directed antibodies. Mol. Pharmacol. 44, 966–971.

Moebius, F.F., Hanner, M., Knaus, H.-G., Weber, F., Striessnig, J., Glossmann, H., 1994. Purification and amino-terminal sequencing of the high affinity phenylalkylamine Ca^{2+} antagonist binding protein from guineas pig liver endoplasmic reticulum. J. Biol. Chem. 269, 29314–29320.

Quirion, R., Bowen, W.D., Itzhak, Y., Junien, J.L., Musacchio, J.M., Rothman, R.B., Su, T.-P., Tam, S.W., Taylor, D.P., 1992. A proposal for the classification of sigma binding sites. Trends Pharmacol. Sci. 13, 85–86.

Schuster, D.I., Ehrlich, G.K., Murphy, R.B., 1994. Purification and partial amino acid sequence of a 28-kDa cyclophilin-like component of the rat liver sigma receptor. Life Sci. 55, 151–156.

Schuster, D.I., Arnold, F.J., Murphy, R.B., 1995. Purification, pharmacological characterization and photoaffinity labeling of sigma receptors from rat and bovine brain. Brain Res. 670, 14–28.

Sonders, M.S., Keana, F.W., Weber, E., 1988. Phencyclidine and psychotomimetic sigma opiates: recent insights into their biochemical and physiological site of action. Trends Neurosci. 11, 37–40.

Su, T.-P., 1991. σ Receptors—putative links between nervous, endocrine and immune systems. Eur. J. Biochem. 200, 633–642.

Su, T.-P., 1993. Delineating biochemical and functional properties of sigma receptors: emerging concepts. Crit. Rev. Neurobiol. 7, 187–203.

Walker, J.M., Bowen, W.D., Walker, F.O., Matsumoto, R.R., de Costa, B., Rice, K., 1990. Sigma receptors: biology and function. Pharmacol. Rev. 42, 355–402.

Weber, E., Sonders, M., Quarum, M., McLean, S., Pou, S., Keana, J.F., 1986. 1,3-Di(2-[5-^{3}H]tolyl)guanidine: a selective ligand that labels sigma-type receptors for psychotomimetic opiates and antipsychotic drugs. Proc. Natl. Acad. Sci. USA 83, 8784–8788.

Wehling, M., 1995. Looking beyond the dogma of genomic steroid action: insights and facts of the 90s. J. Mol. Med. 73, 439–447.

Wehling, M., 1997. Specific, nongenomic actions of steroid hormones. Annu. Rev. Physiol. 59, 365–393.

Yamada, M., Nishigami, T., Nakasho, K., Nishimoto, Y., Miyaji, H., 1994. Relationship between sigma-like site and progesterone-binding site of adult male rat liver microsomes. Hepatology 20, 1271–1280.