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High affinity of sigma$_1$-binding sites for sterol isomerization inhibitors: evidence for a pharmacological relationship with the yeast sterol C_8-C_7 isomerase

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1 The sigma-drug binding site of guinea-pig liver is carried by a protein which shares significant amino acid sequence similarities with the yeast sterol C_8-C_7 isomerase (ERG2 protein). Pharmacologically - but not structurally - the sigma$_1$-site is also related to the emopamil binding protein, the mammalian sterol C_8-C_7 isomerase. We therefore investigated if sterol C_8-C_7 isomerase inhibitors are high affinity ligands for the (+)-[^{3}H]-pentazocine labelled sigma$_1$-binding site.

2 Among the compounds which bound with high affinity to native hepatic and cerebral as well as to yeast expressed sigma$_1$-binding sites were the agricultural fungicide fenpropimorph (K_i 0.005 nM), the antihypocholesterinaemic drugs triparanol (K_i 7.0 nM), AY-9944 (K_i 0.46 nM) and MDL28,815 (K_i 0.16 nM), the enantiomers of the ovulation inducer clomiphene (K_i 5.5 and 12 nM, respectively) and the antioestrogene tamoxifen (K_i 26 nM).

3 Except for tamoxifen these affinities are essentially identical with those for the [^{3}H]-ifenprodil labelled sterol C_8-C_7 isomerase of *S. cerevisiae*. This demonstrates that sigma$_1$-binding protein and yeast isomerase are not only structurally but also pharmacologically related. Because of its affiliations with yeast and mammalian sterol isomerases we propose that the sigma$_1$-binding site is localized on a sterol isomerase related protein, involved in postsqualene sterol biosynthesis.

Keywords: Ergosterol; cholesterol; ERG2; sterol C_8-C_7 isomerase; sigma$_1$-binding site; AY-9944; fenpropimorph; triparanol; tamoxifen; clomiphene

Introduction

The so-called sigma-receptor was originally postulated to account for the psychomimetic effects elicited by benzomorphan opioids such as N-allylnormetazocine and pentazocine (Martin *et al*., 1976). Subsequent studies revealed that sigma-sites were distinct from opiate and NMDA/phencyclidine-receptors (Walker *et al*., 1990). Despite much data on *in vitro* or *in vivo* effects mediated by sigma-sites no physiological or biochemical function was unequivocally shown to be associated with them. In contrast to function, the binding characteristics of sigma-sites are well defined and allow the distinction of two subtypes (Quirion *et al*., 1992). The sigma$_1$- is distinguished from the sigma$_2$-subtype by its higher affinity for the benzomorphan (+)-pentazocine. Several clinically used drugs such as the antipsychotic haloperidol (K_i 0.2 nM), the tricyclic antidepressant opipramol (K_i 0.3 nM) and the opioid analgesic pentazocine (K_i 1.7 nM) interact with sigma$_1$-binding sites with high affinity (Hanner *et al*., 1996). New drugs developed for other receptor systems are also often active in sigma-binding assays. However, the biological consequences of these high affinity interactions are unknown. To clarify the pharmacological significance of drug binding to sigma-sites we isolated and cloned the cDNA of the high affinity (+)-[^{3}H]-pentazocine binding protein (Hanner *et al*., 1996). Its amino acid sequence showed significant similarities with sterol C_8-C_7 isomerases from fungi (Hanner *et al*., 1996). This enzyme is essential for the biosynthesis of ergosterol which is the fungal equivalent of cholesterol.

In mammals cholesterol biosynthesis after cyclization of squalene and formation of lanosterol involves several enzymatic steps such as the saturation of the C24(25) double bond in the side chain, the C14-demethylation, isomerization of the C8(9) double bond to C7(8), introduction of the C5(6) double bond and removal of the C7(8) double bond (Trzaskos *et al*., 1982). Whereas all relevant genes of the yeast sterol pathway have been cloned from *S. cerevisiae* (Lees *et al*., 1995) most of the cDNAs encoding the mammalian enzymes are yet unknown. The recent discovery that the biochemical defect of the inborn Smith-Lemli-Opitz syndrome which is associated with mental retardation and genital malformations results in the accumulation of 7-dehydrocholesterol (Tint *et al*., 1994) and desmosterol (Clayton *et al*., 1996) revealed the pivotal importance of mammalian postsqualene cholesterol biosynthesis.

Some of the compounds interfering with the late steps of cholesterol biosynthesis impair sterol C_8-C_7 isomerization resulting in the accumulation of zymosterol. The striking structural similarities between mammalian sigma$_1$-binding protein and yeast sterol isomerases (Hanner *et al*., 1996) suggested a pharmacological and functional relationship. We now demonstrate that drugs which were shown to interfere with sterol isomerization inhibit (+)-[^{3}H]-pentazocine binding to liver, brain and yeast expressed sigma$_1$-sites with high affinities which are similar to those for the sterol C_8-C_7 isomerase of *S. cerevisiae*. A pharmacological relationship between sigma$_1$-binding site and the mammalian sterol C_8-C_7 isomerase (which is identical with the emopamil binding protein (Moebius *et al*., 1993; Silve *et al*., 1996a)) has already been suggested previously (Moebius *et al*., 1994). Our findings insinuate an affiliation of sigma$_1$-sites with postsqualene cholesterol biosynthesis.

Methods

Binding assays

(±)-[^{3}H]-pentazocine (0.6 nM) or [^{3}H]-ifenprodil (0.6 nM) were incubated in 0.5 or 2.5 ml 25 mM Tris-HCl (pH 9 at 4°C, pH 8.3 at 22°C) for 12 h at 22°C with 2 to 35 μg ml^{-1} microsomal protein. Non-specific binding was measured in the presence of

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1 μM of unlabelled drug. Serial dilutions of competing drugs were prepared in dimethylsulphoxide (Boer *et al*., 1989) and added directly to the assay. The final dimethylsulphoxide (DMSO) concentration was $\leqslant 1\%$, which did not affect specific binding. For the separation of bound and free ligand we used Whatman GF/C filters presoaked in 0.3% (w/v) polyethyleneimine. Filters were washed with icecold 10 mM Tris-HCl (pH 9 at 4°C). Binding parameters were calculated by non linear curve fitting to a rectangular hyberbola (K_d (dissociation constant), B_{max} (maximal density of binding sites)) or the general dose-response equation (IC_{50} (concentration causing half-maximal inhibition), slope factors, (DeLean *et al*., 1978)). K_i (inhibition constants) values were calculated according to Linden (1982).

Membrane preparation

Microsomes from yeast strain WA0 (a his7-2 leu2-3,112 ura3-52 erg2-3) overexpressing the sigma$_1$-binding site (6 × HIS-λGP8-ORF, (Hanner *et al*., 1996)) and from strain JB811 (ade2-1 leu2-3,112 pep4-3 trpl-289 ura3-52) were prepared as described by Moebius *et al*. (1996). Guinea-pig liver and whole brain microsomes were prepared by homogenization with a glass-Teflon homogenizer in ice-cold 0.25 M sucrose, 10 mM Tris-HEPES pH 7.4 (4°C). The homogenate was centrifuged at 8,000 × *g* and the resulting supernatant was collected by centrifugation at 100,000 × *g*. The pellet was resuspended in 0.5 M KCl, 0.15 M Tris-HCl pH 8.0 (4°C) and centrifuged at 100,000 × *g*. The final pellet was resuspended in 5% (w/v) glycerol, 20 mM Tris-HCl pH 9 (4°C) at a protein concentration of 4 to 8 mg ml^{-1}, shock-frozen in liquid nitrogen and stored at −80°C. Protein concentrations were determined according to Bradford (1976), with bovine serum albumin (BSA) as a standard.

Materials

(+)-[^{3}H]-pentazocine (32 Ci mmol^{-1}) and [^{3}H]-ifenprodil (44 Ci mmol^{-1}) were obtained from NEN (Vienna, Austria). Chemicals were obtained from the following sources: zuclomiphene, enclomiphene, triparanol and MDL28,815 (M-[1,5,9)-trimethyldecyl]-4,10-dimethyl-8-aza-trans-decal-3β-ol) Hoechst Marion Roussel Research Institute (Cincinnati, OH); L-690,404 (1-butyl-3,4-dihydrospiro[naphtalene-1-(2H),4′piperidine]) Merck Sharp & Dohme (Harlow, U.K.); fenpropimorph and tridemorph, BASF (Limburgerhof, Germany); AY-9944 (1,4-bis-(2-chlorobenzylaminomethyl)cyclohexane) Dr P. Benveniste (Strasbourg, France); trifluperidol, RBI (Natick, MA); Bradford Protein Reagent, Bio-Rad (Vienna, Austria). All other chemicals were obtained from Sigma (Vienna, Austria). *S. cerevisiae* strain WA0 was kindly provided by Dr M. Bard (Indianapolis, IN, U.S.A.); strain JB811 was from Dr K. Nasmyth (Vienna, Austria).

Results

We examined the interaction of inhibitors of sterol isomerization with native sigma$_1$-binding sites in guinea-pig brain and liver microsomes as well as with the sigma$_1$-binding site expressed in *S. cerevisiae* strain WA0 (Ashman *et al*., 1991) which is devoid of sterol C_8-C_7 isomerase activity. Although (+)-[^{3}H]-pentazocine is a sigma$_1$-selective ligand which has low affinity for the endogenous sigma-ligand binding site of *S. cerevisiae* associated with the sterol C_8-C_7 isomerase (ERG2 gene product) (Moebius *et al*., 1996) we wanted to rule out any interference with endogenous isomerase. Transformation with the cDNA encoding the sigma$_1$-receptor (6 × HIS-λGP8-ORF, (Hanner *et al*., 1996)) induced the formation of high affinity (+)-[^{3}H]-pentazocine binding sites (Figure 1a) which were sensitive to haloperidol and ditolylguanidine (Figure 1b). The expressed (+)-[^{3}H]-pentazocine binding sites also showed high affinities for L-609,404, a spiropiperidine, (Table 1, Figure 1b) (Chambers *et al*., 1992) and amiodarone, an antiarrhythmic drug which is also a sigma-ligand (Cagnotto *et al*., 1994) (K_i 1.7 ± 0.5 nM ($n = 3$)).

Inhibitors of fungal ergosterol biosynthesis

The haloperidol analogue trifluperidol is clinically used as an antipsychotic. Besides its prominent antidopaminergic activity trifluperidol also inhibits the yeast sterol C_8-C_7 iso-

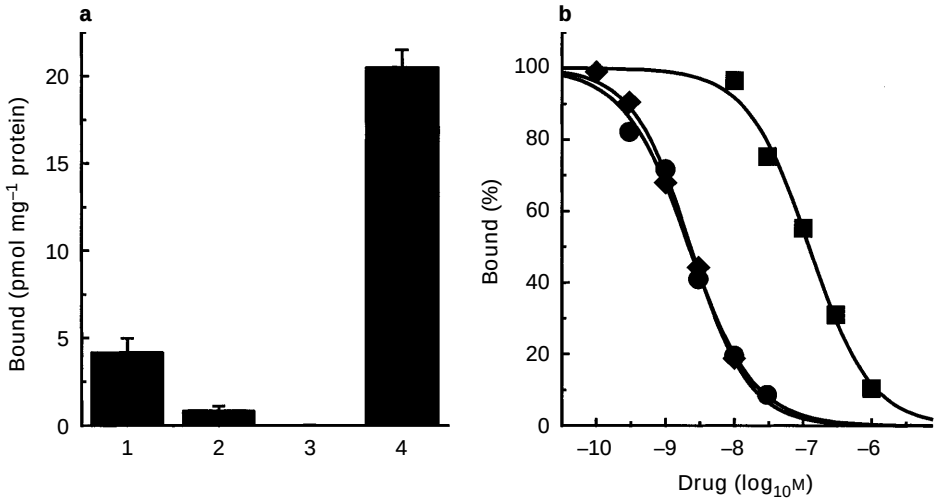

Figure 1 Characterization of (+)-[^{3}H]-pentazocine binding to the recombinant sigma$_1$-binding site. (a) Binding of 0.6 nM (+)-[^{3}H]-pentazocine to microsomes from guinea-pig liver (1), brain (2) and yeast cells (strain WA0, (Ashman *et al*., 1991)) transformed with the vector (3) or the 6 × HIS-λGP8-ORF DNA (Hanner *et al*., 1996) (4). Data shown are the mean ± s.d. of three experiments. (b) Inhibition of (+)-[^{3}H]-pentazocine (0.6 nM) binding to the yeast expressed sigma$_1$-binding site (4 μg microsomal protein ml^{-1}) by (±)-pentazocine (●, IC_{50} 2.2 nM), ditolylguanidine (■, IC_{50} 122 nM) and L-609,404 (◆, IC_{50} 2.3 nM). Data shown are the mean of duplicate determinations.

merase (Sobus *et al*., 1977). It differs from haloperidol by a trifluoromethyl group instead of a chlorine atom and has modestly lower affinity (K_i 0.83±0.24 nM ($n=3$)) than haloperidol (K_i 0.2 nM (Hanner *et al*., 1996)) for the liver (+)-[^{3}H]-pentazocine binding site (Table 1, Figure 2a). Fenpropimorph is an agricultural fungicide which inhibits the yeast sterol isomerase (Marcireau *et al*., 1990). With K_i values for the liver and brain (+)-[^{3}H]-pentazocine binding sites of 0.011±0.006 nM ($n=3$) and 0.005±0.004 nM ($n=3$), respectively, fenpropimorph is a sigma$_1$-ligand with the highest affinity found so far. Its analogue tridemorph displayed similarly high affinity for the brain binding site (K_i 0.023±0.005 nM ($n=3$)). Because of this extraordinarily high affinity the K_i determination critically depends on the receptor concentration (Knaus *et al*., 1995). Indeed when the IC_{50} values were determined under standard conditions for liver, brain and yeast expressed (+)-[^{3}H]-pentazocine binding sites ($IC_{50}=0.10\pm0.01$ nM ($n=3$, 0.10±0.03 nM ($n=3$) and 0.31±0.11 nM ($n=3$), respectively) they corresponded to half of the receptor concentration (0.23±0.04, 0.18±0.03 and 0.54±0.11 nM, respectively). Apparent Hill slopes significantly different from unity (data not shown) also suggested that the number of receptors exceeded the number of drug molecules (Knaus *et al*., 1995). Reduction of receptor concentration and an increased assay volume (2.5 instead of 0.5 ml) remedied these problems (Figure 2a, Table 1).

Inhibitors of postsqualene cholesterol biosynthesis

MDL28,815, a rationally designed inhibitor of sterol biosynthesis, resembles cholesterol and mimics the high energy reaction intermediates of several of the late steps of sterol modification (Gerst *et al*., 1988). Despite a chemical structure different from that of morpholines its affinity for the (+)-[^{3}H]-pentazocine labelled sigma$_1$-site of guinea-pig brain microsomes was close (K_i 0.16±0.04 nM ($n=3$)) to that of tridemorph (Table 1). The structure of the antihypercholesteraemic drug AY-9944 differs from fenpropimorph and MDL28,815. AY-9944 was therefore employed to confirm further that the high affinity interaction of sterol isomerase inhibitors with the sigma$_1$-site did not simply reflect a chemical relationship. Indeed AY-9944, which is a symmetric molecule with two nitrogen atoms, displayed high affinity for the (+)-[^{3}H]-pentazocine labelled sigma$_1$-binding site in brain (K_i 0.46±0.10 nM). Triparanol was developed for the treatment of hypercholesterinaemia. It showed high affinity for the (+)-[^{3}H]-pentazocine labelled brain sigma$_1$-binding site (K_i 7.0±0.8 nM ($n=3$)). Several other compounds which are

Table 1 Comparison of the inhibition of (+)-[^{3}H]-pentazocine binding to native and heterologously expressed sigma$_1$-sites and to the [^{3}H]-ifenprodil binding site of the yeast sterol C_8-C_7 isomerase

	(+)-[^{3}H]-pentazocine						*[^{3}H]-ifenprodil*
	Liver		*Brain*		*Yeast Expressed*		*S. cerevisiae*
	K_i (nM)	*Slope*	K_i (nM)	*Slope*	K_i (nM)	*Slope*	K_i (nM)
σ-Ligands							
L-609,404	0.63±0.08	1.02±0.12	0.60±0.11	0.93±0.02	1.3±0.4	0.93±0.08	4.7±0.6
Amiodarone	1.4±0.2	1.07±0.06	2.1±0.7	0.95±0.15	1.7±0.5	0.90±0.03	62[a]
Isomerase inhibitors							
Trifluperidol	0.83±0.24	0.82±0.04	1.3±0.1	0.93±0.17	3.6±1.3	0.94±0.09	0.15[a]
Fenpropimorph	0.011±0.006	1.07±0.25	0.005±0.004	1.01±0.05	0.08±0.02	0.98±0.10	0.05[a]
Tridemorph	0.039±0.023	1.07±0.17	0.023±0.005	1.13±0.15	0.10±0.04	1.11±0.03	0.09[a]
MDL28,815	0.48±0.17	1.65±0.28	0.16±0.04	1.46±0.32	0.49±0.11	1.27±0.23	0.44[a]
AY-9944	0.50±0.10	1.39±0.14	0.46±0.10	1.27±0.22	0.99±0.12	1.20±0.09	5.8[a]
Triparanol	8.2±0.6	1.09±0.02	7.0±0.8	0.92±0.10	15±2	1.07±0.06	1.5[a]
Tamoxifen	34±3	1.27±0.08	26±5	0.84±0.05	54±12	1.16±0.20	1,470±95
Zuclomiphene	4.7±0.6	1.06±0.15	5.5±0.4	1.05±0.07	12±2	1.08±0.03	1.6±0.4
Enclomiphene	7.7±0.5	0.93±0.13	12±2	0.87±0.03	17±4	0.99±0.03	164±26

Binding conditions are given in Methods. Data shown are the mean±s.d. ($n=3$). [a] Data taken from Moebius *et al*. (1996).

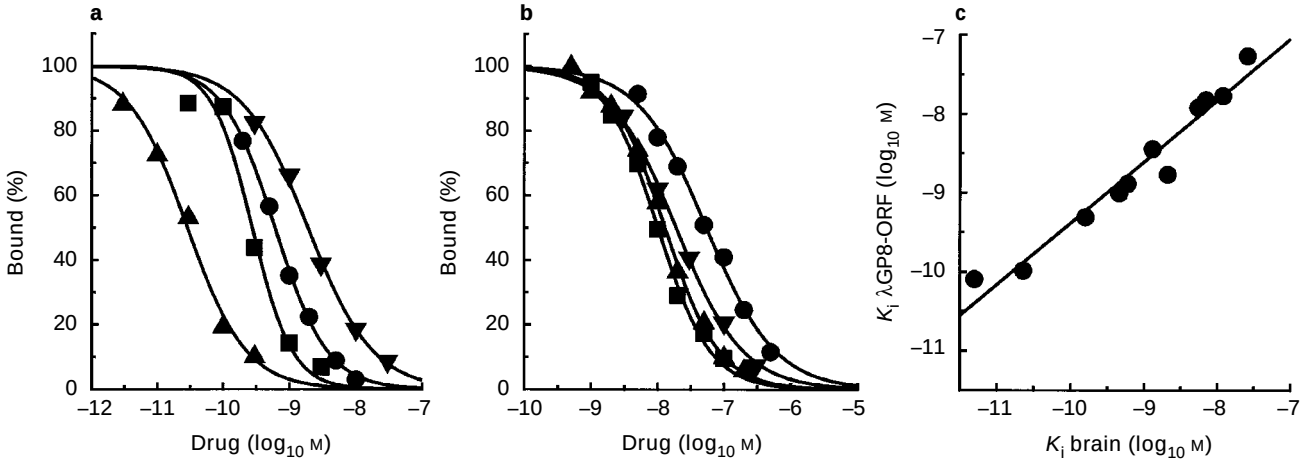

Figure 2 Inhibition of (+)-[^{3}H]-pentazocine binding by sterol isomerase inhibitors. (a) (+)-[^{3}H]-pentazocine 0.6 nM was incubated with brain microsomes in a final volume of 0.5 ml (fenpropimorph 2.5 ml) and the inhibition by fenpropimorph (▲), MDL28,815 (■), AY-9944 (●) and trifluperidol (▼), and (b) triparanol (▲), zuclomiphene (■), enclompiphene (▼) and tamoxifen (●) was measured. Data shown are the mean of duplicate determinations. (c) Comparison of the K_i values (Table 1) obtained for the brain (x-axis) and the yeast expressed (y-axis) (+)-[^{3}H]-pentazocine binding sites ($r=0.98$).

structurally related to triparanol were also tested. The racemic mixture of the oestrogen receptor antagonist tamoxifen showed high affinity (K_i 26 ± 5 nM ($n = 3$)). This binding was not stereoselective as shown by the essentially identical affinities of the *cis*- and *trans*-stereoisomers of the tamoxifen analogue clomiphene, zuclomiphene and enclomiphene (K_i 5.5 ± 0.4 nM ($n = 3$) and 12 ± 2 nM ($n = 3$), respectively).

The affinities for the native and expressed (+)-[^{3}H]-pentazocine binding site correlate

The cDNA 6 × HIS-λGP8-ORF (Hanner *et al*., 1996) induces the formation of a high affinity binding site with all characteristics of the native brain and liver sigma$_1$-binding sites. Therefore, we compared the affinities of the isomerase inhibitors for native and recombinant (+)-[^{3}H]-pentazocine labelled sites. In agreement with previous findings (Hanner *et al*., 1996) the affinity of the heterologously expressed protein for most compounds is slightly reduced. This might be due to differences in the lipid composition between yeast and guinea-pig microsomes (Hanner *et al*., 1996). However, we found an excellent correlation between the K_i values for the guinea-pig brain and the yeast expressed (+)-[^{3}H]-pentazocine binding sites ($r = 0.98$, Figure 2c). This strengthens our claim that the recently cloned cDNA (Hanner *et al*., 1996) encodes the sigma$_1$-site of guinea-pig liver and brain.

Discussion

Pharmacological similarities of the sigma$_1$-site with the yeast sterol isomerase

The guinea-pig sigma$_1$-binding protein shares striking amino acid sequence similarities with the ERG2 gene product of *S. cerevisiae* (Hanner *et al*., 1996) which encodes a sterol C_8-C_7 isomerase (Ashman *et al*., 1991). The ERG2 protein also carries a high affinity binding site for the sigma-ligands [^{3}H]-ifenprodil and [^{3}H]-haloperidol but not for (+)-[^{3}H]-pentazocine (Moebius *et al*., 1996) in line with the inhibition of the yeast sterol C_8-C_7 isomerase by the sigma-ligand SR31747 (Silve *et al*., 1996b). We now demonstrate that both proteins not only share structural but also pharmacological properties. Several structurally diverse drugs show only minor differences in their affinity for the [^{3}H]-ifenprodil labelled sterol C_8-C_7 isomerase of *S. cerevisiae* and the mammalian (+)-[^{3}H]-pentazocine binding site (Table 1). For tridemorph, MDL28,815 and zuclomiphene this difference was <5 fold, for L-690,404, AY-9944, fenpropimorph, triparanol and enclomiphene it was <15 fold and only tamoxifen showed a >50 fold reduced affinity for the yeast [^{3}H]-ifenprodil binding site as compared to the sigma$_1$-site. Interestingly the yeast sterol C_8-C_7 isomerase preferred zuclomiphene (K_i 1.6 nM) to enclomiphene (K_i 164 nM) whereas the sigma$_1$-site was essentially not stereoselective for clomiphene. The structural and pharmacological similarities of mammalian sigma$_1$-binding protein and yeast sterol C_8-C_7 isomerase suggests that both proteins originated from a common precursor.

Sterol isomerase inhibitors: a new class of sigma-ligands

Fenpropimorph and tridemorph are agricultural fungicides which inhibit sterol C_8-C_7 isomerases of fungi and plants (Schmitt *et al*., 1981; Rahier *et al*., 1986; Marcireau *et al*., 1990). The ultrahigh affinity of fenpropimorph for the brain α_1-site (K_i 5 pM) suggests that this morpholine might be a valuable tool for studies on sigma$_1$-mediated effects. The haloperidol congener trifluperidol (K_i 1.3 nM) inhibits the sterol C_8-C_7 isomerase of yeast (Sobus *et al*., 1977) whereas the azadecalin MDL28,815 (K_i 0.16 nM) inhibits the mammalian sterol C_8-C_7 isomerase in 3T3 mouse fibroblasts (Gerst *et al*., 1988) as well as in the human hepatoma cell line HepG2 (van Sickle *et al*., 1993). The anti-hypercholesteraemic drug AY-9944 (K_i 0.46 nM) reduces sterol C_8-C_7 isomerization in yeast (Pereira *et al*., 1983) and rat adrenal gland (Givner *et al*., 1967). It also inhibits the rat sterol C_8-C_7 isomerase *in vitro* (Paik *et al*., 1986). Triparanol (K_i 7.0 nM) was used in man for the treatment of hypercholesterinaemia but was withdrawn from marketing because of cataract induction (Kirby *et al*., 1962). In yeast and rat hepatoma cells triparanol is an inhibitor of sterol C_8-C_7 isomerization (Campagnoni *et al*., 1977; Popják *et al*., 1989). Clomiphene induces ovulation and is used to treat female sterility. Its stereoisomer zuclomiphene (K_i 5.5 nM) inhibits the sterol C_8-C_7 isomerase of the developing rat brain (Ramsey *et al*., 1977). Tamoxifen (K_i 26 nM) is an antioestrogenic drug which is used to treat hormone-dependent breast cancer. Sterol C_8-C_7 isomerase activity is compromised in tamoxifen treated women (Gylling *et al*., 1995).

The propensity of sigma-sites to bind chemically diverse drugs from various pharmacological classes such as antidepressants, opioids and antipsychotics is well known. However, α-sites fail to bind all representatives of the respective pharmacological class of drugs. For example their affinity for the dopamine D_2-antagonists haloperidol and chlorpromazine is high whereas it is low for risperidone (Leysen *et al*., 1992), sulpiride and spiperone (Walker *et al*., 1990). We therefore consider sterol isomerase inhibitors to be the first class of sigma-ligands with diverse chemical structures but a common biochemical effect. It remains to be clarified whether *vice versa* all sigma$_1$-ligands are inhibitors of sterol C_8-C_7 isomerization in brain which contains high densities of α_1-sites.

Conclusion

Blockers of eukaryontic sterol C_8-C_7 isomerization potently inhibit (+)-[^{3}H]-pentazocine binding to sigma-sites. Their affinities are similar to those for the sterol C_8-C_7 isomerase of *S. cerevisiae* except for tamoxifen. Despite its structural and pharmacological relationship with the yeast isomerase the overexpressed α_1-binding protein failed to complement the sterol C_8-C_7 isomerization deficiency of yeast mutants (Hanner *et al*., 1996). It is therefore unclear whether the protein carrying the sigma$_1$-site is not only a structural homologue and a pharmacological relative but also a functional equivalent of the yeast isomerase. A mammalian sterol C_8-C_7 isomerase cloned by complementation of an isomerase deficient yeast strain (Silve *et al*., 1996a) is identical with the emopamil binding protein (Zech *et al*., 1991; Moebius *et al*., 1993; 1994; Hanner *et al*., 1995). The latter is pharmacologically related to the sigma$_1$-site although it bears no structural similarities. The pharmacological and structural affiliations of sigma$_1$-sites with yeast and mammalian sterol C_8-C_7 isomerases imply a related function. We therefore propose that the α_1-binding site is carried by a sterol isomerase-related protein involved in post-squalene cholesterol biosynthesis. Its precise biochemical function remains to be determined by targeted disruption of the murine gene.

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References

ASHMAN, W.H., BARBUCH, R.J., ULBRIGHT, C.E., JARRETT, H.W. & BARD, M. (1991). Cloning and disruption of the yeast C-8 sterol isomerase gene. *Lipids*, **26,** 628 – 632.

BOER, R., GRASSEGGER, A., SCHUDT, C. & GLOSSMANN, H. (1989). (+)-Niguldipine binds with very high affinity to Ca^{2+} channels and to a subtype of alpha$_1$-adrenoceptors. *Eur. J. Pharmacol.*, **172,** 131 – 145.

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.

CAGNOTTO, A., BASTONE, A. & MENNINI, T. (1994). [^{3}H]-pentazocine binding to rat brain sigma$_1$ receptors. *Eur. J. Pharmacol.*, **266,** 131 – 138.

CAMPAGNONI, C., HOLMLUND, C.E. & WHITTAKER, N. (1988). The effect of triparanol on the composition of free and esterified sterols of *Saccharomyces cerevisiae*. *Arch. Biochem. Biophys.*, **184,** 555 – 560.

CHAMBERS, M.S., BAKER, R., BILLINGTON, D.C., KNIGHT, A.K., MIDDLEMISS, D.N. & WONG, E.H.F. (1992). Spiropiperidines as high-affinity, selective sigma ligands. *J. Med. Chem.*, **35,** 2033 – 2039.

CLAYTON, P., MILLS, K., KEELING, J. & FITZPATRICK, D. (1996). Desmosterolosis: a new inborn error of cholesterol biosynthesis. *Lancet*, **348,** 404.

DELEAN, A., MUNSON, P.J. & RODBARD, D. (1978). Simultaneous analysis of families of sigmoid curves: application to bioassays, radioligand assay and physiological dose-response curves. *Am. J. Physiol.*, **4,** E97 – E102.

GERST, N., DURIATTI, A., SCHUBER, F., TATON, M., BENVENISTE, P. & RAHIER, A. (1988). Potent inhibition of cholesterol biosynthesis in 3T3 fibroblasts by N-[(1,5,9)-trimethyldecyl]-4alpha,10-dimethyl-8-aza-trans-decal-3beta-ol, a new 2,3-oxidosqualene cyclase inhibitor. *Biochem. Pharmacol.*, **37,** 1955 – 1964.

GIVNER, M.L., ROCHFORT, J.G. & DVORNIK, D. (1967). Effect of AY-9944 on rat adrenal function. *Endocrinology*, **81,** 976 – 980.

GYLLING, H., PYRHÖNEN, S., MÄNTYLÄ, E., MÄENPÄÄ, H., KANGAS, L. & MIETTINEN, T.A. (1995). Tamoxifen and toremifene lower serum cholesterol by inhibition of delta8-cholestenol conversion to lathosterol in women with breast cancer. *J. Clin. Oncol.*, **13,** 2900 – 2905.

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. U.S.A.*, **93,** 8072 – 8077.

HANNER, M., MOEBIUS, F.F., WEBER, F., GRABNER, M., STRIESSNIG, J. & GLOSSMAN, H. (1995). Phenylalkylamine Ca^{2+} antagonist binding protein. Molecular cloning, tissue distribution, and heterologous expression. *J. Biol. Chem.*, **270,** 7551 – 7557.

KIRBY, T.J., ACHOR, R.W.P., PERRY, H.O. & WINKELMANN, R.K. (1962). Cataract formation after triparanol therapy. *Arch. Ophtalmol.*, **68,** 486 – 489.

KNAUS, H.-G., KOCH, R.O.A., EBERHART, A., KACZOROWSKI, G.J., GARCIA, M.L. & SLAUGHTER, R.S. (1995). [^{125}I]-Margatoxin, an extraordinarily high affinity ligand for voltage-gated potassium channels in mammalian brain. *Biochemistry*, **34,** 13627 – 13634.

LEES, N.D., SKAGGS, B., KIRSCH, D.R. & BARD, M. (1995). Cloning of the late genes in the ergosterol biosynthetic pathway of saccharomyces cerevisiae - a review. *Lipids*, **30,** 221 – 226.

LEYSEN, J.E., JANNSEN, P.M.F., GOMMEREN, W., WYNANTS, J., PAUWELS, P.J. & JANSSEN, P.A.J. (1992). *In vitro* and *in vivo* receptor binding and effects on monoamine turnover in rat brain regions of the novel antipsychotics risperidone and ocaperidone. *Mol. Pharmacol.*, **41,** 494 – 508.

LINDEN, J. (1982). Calculating the dissociation constant of an unlabeled compound from the concentration required to displace radioactive binding by 50%. *J. Cyclic Nucleotide Res.*, **8,** 163 – 172.

MARCIREAU, C., GUILLOTON, M. & KARST, F. (1990). In vivo effects of fenpropimorph on the yeast *Saccharomyces cerevisiae* and determination of the molecular basis of the antifungal property. *Antimicrob. Agents Chemother.*, **34,** 989 – 993.

MARTIN, W.R., EADES, C.E., THOMPSON, J.A., HUPPLER, R.E. & GILBERT, P.E. (1976). The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. *J. Pharmacol. Exp. Ther.*, **197,** 517 – 532

MOEBIUS, F.F., BURROWS, G.G., STRIESSNIG, J. & GLOSSMANN, H. (1993). Biochemical characterization of a 22-kDa high affinity antiischemic drug-binding polypeptide in the endoplasmic reticulum of guinea pig liver: potential common target for antiischemic drug action. *Mol. Pharmacol.*, **43,** 139 – 148.

MOEBIUS, F.F., HANNER, M., KNAUS, H.-G., WEBER, F., STRIESSNIG, J. & GLOSSMANN, H. (1994). Purification and N-terminal sequencing of the high affinity phenylalkylamine Ca^{2+} antagonist binding protein from guinea-pig liver endoplasmic reticulum. *J. Biol. Chem.*, **269,** 29314 – 29320.

MOEBIUS, F.F., BERMOSER, K., REITER, R.J., HANNER, M. & GLOSSMANN, H. (1996). Yeast sterol $C_8 - C_7$ isomerase: identification and characterization of a high affinity binding site for enzyme inhibitors. *Biochemistry*, **35,** 16871 – 16878.

PAIK, Y.-K., BILLHEIMER, J.T., MAGOLDA, R.L. & GAYLOR, J.L. (1986). Microsomal enzymes of cholesterol biosynthesis from lanosterol. Solubilization and purification of steroid 8-isomerase. *J. Biol. Chem.*, **261,** 6470 – 6477.

PEREIRA, R., HOLMLUND, C.E. & WHITTAKER, N. (1983). The effect of AY-9944 on yeast sterol and sterol ester metabolism. *Lipids*, **18,** 545 – 552.

POPJÁK, G., MEENAN, A., PARISH, E.J. & NES, W.D. (1989). Inhibition of cholesterol synthesis and cell growth by 24(R,S), 25-iminolanosterol and tripanol in cultured rat hepatoma cells. *J. Biol. Chem.*, **264,** 6230 – 6238.

QUIRION, R., BOWEN, W.D., ITZHAK, Y., JUNIEN, J.L., MUSACCHIO, J.M., ROTHMAN, R.B., SU, T.-P., TAM, W.S. & TAYLOR, D.P. (1992). A proposal for the classification of sigma binding sites. *Trends Pharmacol. Sci.*, **13,** 85 – 86.

RAHIER, A., SCHMITT, P., HUSS, B., BENVENISTE, P. & POMMER, E.H. (1986). Chemical structure-activity relationships of the inhibition of sterol biosynthesis by n-substituted morpholines in higher plants. *Pestic. Biochem. Phys.*, **25,** 112 – 124.

RAMSEY, R.B., FREDERICKS, M. & FISCHER, V.M. (1977). Effect of zuclomiphene on cholesterol formation in the developing central nervous system of the rat. *J. Neurochem.*, **28,** 1317 – 1321.

SCHMITT, P., BENVENISTE, P. & LEROUX, P. (1981). Accumulation of 9 beta, 19-cyclopropyl sterols in suspension cultures of bramble cells cultured with tridemorph. *Phytochemistry*, **20,** 2153 – 2159.

SILVE, S., DUPUY, P.H., LABIT-LEBOUTEILLER, C., KAGHAD, M., CHALON, P. RAHIER, A., TATON, M., LUPKER, J., SHIRE, D. & LOISON G. (1996a). Emopamil-binding protein, a mammalian protein that binds a series of structurally diverse neuroprotective agents, exhibits delta8-delta7 isomerase activity in yeast. *J. Biol. Chem.*, **271,** 22434 – 22440.

SILVE, S., LEPLATOIS, P., JOSSE, A., DUPUY, P.H., LANAU, C., KAGHAD, M., DHERS, C., PICARD, C., RAHIER, A., TATON, M., LEFUR, G., CAPUT, D., FERRARA, P. & LOISON, G. (1996b). The immunosuppressant SR31747 blocks cell proliferation by inhibiting a steroid isomerase in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, **16,** 2719 – 2727.

SOBUS, M.T., HOLMLUND, C.E. & WHITTAKER, N.F. (1977). Effects of the hypocholesteremic agent trifluperidol on the sterol, steryl ester and fatty acid metabolism of *Saccharomyces cerevisiae*. *J. Bacteriol.*, **130,** 1310 – 1316.

TINT, G.S., IRONS, M., ELIAS, E.R., BATTA, A.K., FRIEDEN, R., CHEN, T.S. & SALEN, G. (1994). Defective cholesterol biosynthesis associated with the Smith-Lemli-Opitz syndrome. *N. Engl. J. Med.*, **330,** 107 – 113.

TRZASKOS, J.M., BOWEN, W.D., FISHER, G.J., BILLHEIMER, J.T. & GAYLOR, J.L. (1982). Microsomal enzymes of cholesterol biosynthesis from lanosterol: a progress report. *Lipids*, **17,** 250 – 256.

VAN SICKLE, W.A., WILSON, P.K., WANNAMAKER, M.W., COOPER, J.R., FLANAGAN, M.A., MCCARTHY, J.R., BEY, P. & JACKSON, R.L. (1993). An alternative mechanism for the inhibition of cholesterol biosynthesis in hepG2 cells by n-[(1,5,9)-trimethyldecyl] - 4alpha, 10 - dimethyl - 8 - aza - trans-decal-3betha-ol (mdl28, 815). *J. Pharmacol. Exp. Ther.*, **267,** 1243 – 1249.

WALKER, J.M., BOWEN, W.D., WALKER, F.P., MATSUMOTO, R.R., DE COSTA, B. & RICE, K.C. (1990). Sigma receptors: biology and function. *Pharmacol. Rev*., **42,** 355–402.

ZECH, C., STAUDINGER, R., MÜHLBACHER, J. & GLOSSMANN, H. (1991). Novel sites for phenylalkylamines: characterization of a sodium-sensitive drug receptor with (−)-[^{3}H]emopamil. *Eur. J. Pharmacol*., *208,* 119–130.

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