JPP Journal of Pharmacy and Pharmacology

JPP 2005, 57: 75–81

Received July 22, 2004
Accepted September 21, 2004
DOI 10.1211/0022357055074
ISSN 0022-3573

Comparison of the binding affinity of some newly synthesized phenylethanolamine and phenoxypropanolamine compounds at recombinant human β- and α_1-adrenoceptor subtypes

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Abstract

We evaluated six new compounds, SWR-0065HA ([4-[2-[3-[[(3,4-dihydro-4-oxo-[1,2,4]-triazino(4,5-a)indol)-lyl]oxy]-2-hydroxypropylamino]ethoxy]phenyl]acetic acid methyl ester hydrochloride), SWR-0098NA ((R*R*-UE)-(E)-[4-[3-[(2-phenyl-2-hydroxyethyl)amino]-1-butenyl]phenoxy]acetic acid sodium salt), SWR-0315NA ((E, Z)-[4[[1-[2-[(3-phenoxy-2-hydroxy propyl)]amino]ethyl]-1-propenyl]phenoxy]acetic acid sodium), SWR-0338SA ((E)-[4-[5-[(2-phenyl-2-hydroxyethyl)amino]-2-pentene-3-yl]phenoxy] acetic acid ethanedioic acid), SWR-0342SA ((S)-(Z)-[4-[[1-[2-[(2-hydroxy-3-phenoxypropyl)]amino] ethyl]-1-propenyl]phenoxy]acetic acid ethanedioic acid) and SWR-0345HA ((E)-2-methyl-3-[4-[2-(2-phenyl-2-hydroxyethylamino)ethoxy]phenyl]-2-propenoic acid ethyl ester hydrochloride) for their potencies as selective ligands at human β-adrenoceptors expressed in COS-7 cells and compared the binding affinities for human α_1-adrenoceptors expressed in Chinese hamster ovary (CHO) cells using a radioligand-binding assay. Phenoxypropanolamine derivatives SWR-0315NA and SWR-0342SA showed higher binding affinities for β-adrenoceptor subtypes; SWR-0065HA, however, showed a higher affinity for only β_2-adrenoceptors, accounting for 3-fold and 6-fold selectivity against β_1- and β_3-adrenoceptors. Compounds SWR-0315NA and SWR-0342SA did not show any binding selectivity for any of the subtypes. However, functionally these two compounds are selective for β_3-adrenoceptors. Among the phenylethanolamine derivatives, SWR-0338SA and SWR-0345HA showed 9-fold and 16-fold higher binding selectivity for β_3-adrenoceptors against β_1-adrenoceptors, respectively, whereas they both showed a 7-fold higher binding selectivity for β_3-adrenoceptors against β_2-adrenoceptors. SWR-0098NA did not show any significant binding affinity for any of the β-adrenoceptor subtypes. These compounds, except for SWR-0098NA, were not found to possess any significant binding affinity for α_1-adrenoceptor subtypes over that for β-adrenoceptor subtypes. However, SWR-0098NA has about a 3-fold to 22-fold higher binding selectivity for α_1-adrenoceptor subtypes against β-adrenoceptor subtypes, making it difficult for use in a β-adrenoceptor receptor study. Compounds SWR-0315NA and SWR-0342SA have similar binding potency for α_1-adrenoceptors as adrenaline (epinephrine), proving the finding of this manuscript that this phenoxypropanolamine group of β-adrenoceptor ligands could also be used as α_1-adrenoceptor ligands. Functional assays have to be performed to confirm their agonistic activity.

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Funding: This research was supported by a grant from the Promotion and Mutual Aid Corporation for Private Schools of Japan.

Introduction

The β-adrenoceptors and α_1-adrenoceptors belong to a large family of G-protein-coupled receptors (GPCRs) that are characterized by seven transmembrane helices. They mediate the functional effects of catecholamines like adrenaline (epinephrine) and noradrenaline (norepinephrine) by coupling to several of the major signalling pathways modulated by G proteins.

The β-adrenoceptors were initially divided into β_1- and β_2-adrenoceptors (Lands et al 1967). Further experimentation revealed another receptor subtype, insensitive to β-adrenoceptor antagonists, which was later termed the β_3-adrenoceptor (Arch et al 1984). Another subtype, the β_4-adrenoceptor, has also been reported in the mammalian heart (Kaumann 1997) and human adipose tissue (Galitzky et al 1998), but later, this

subtype was declared a novel state of the β_1-adrenoceptor (Kompa & Summers 1999; Grannemann et al 2001; Lewis et al 2004). The basic structures of β-adrenoceptor ligands are phenylethylamine and phenoxypropanolamine (Nagatomo et al 2001). Phenylethanolamines consist of a benzene ring and an ethylamine side chain, which contains a hydroxyl group at the β-carbon. Most of these compounds (adrenaline, isoproterenol, BRL 37344, etc.) exhibit agonistic properties towards β_1-, β_2-, and β_3-adrenoceptors. The phenoxypropanolamines consist of a phenoxy group attached to a β-hydroxypropanolamine side chain. All of these compounds show antagonism towards β_1- and β_2-adrenoceptors, but they are agonists of β_3-adrenoceptors. Only a few compounds (ICI 118551, CGP 20712A, bupranolol, etc.) exhibit β_3-adrenoceptor antagonistic activity. There are, however, a few exceptions and those compounds are dichloroisoprenaline (DCI), pronethalol, sotalol, etc., which possess the ethanolamine side chain, but show antagonistic activity towards β_1- and β_2-adrenoceptors.

The concept of subtypes of the α_1-adrenoceptor was first suggested in the mid 1980s on the basis of different affinities for the agonist, oxymetazoline, and the antagonists, WB4101 and phentolamine, on certain α_1-adrenoceptor-mediated pharmacological preparations. Subsequent characterization of the α_1-adrenoceptor using radioligand-binding and functional studies has led to the identification of three native prazosin-high-affinity α_1-adrenoceptor subtypes designated α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors, corresponding to the three subtypes (α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors) characterized by molecular cloning techniques (Langer 1999). Muramatsu et al (1990) suggested that there is another α_1-adrenoceptor subtype that has lower affinity for prazosin in addition to those that have higher affinity for prazosin. This new functional subtype was termed the α_{1L}-adrenoceptor. There are two major classes of α-adrenoceptor agonists: the β-phenethylamines, which include compounds such as noradrenaline, phenylephrine and methoxamine and the imidazolines, which include compounds such as clonidine and oxymetazoline. Generally, agonist activity at the α-adrenoceptor decreases with increasing size of the N-substituent in catecholamine-type molecules, while β-activity is often enhanced by the same substitution.

The radioligand-binding assay is a relatively simple but powerful tool for studying G-protein-coupled receptors. Some newly synthesized β_3-adrenoceptor agonists, such as SWR-0065HA, SWR-0098NA, SWR-0315NA, SWR-0338SA, SWR-0342SA and SWR-0345HA, have been investigated for their affinity for β-adrenoceptor subtypes and α_1-adrenoceptor subtypes by radioligand-binding assay. Affinities of these SWR-compounds for β_3-adrenoceptor subtype have been discussed in our previous article (Ahmed et al 2003). SWR-0315NA has also been proved to be a β_3-adrenoceptor subtype selective drug in a second messenger accumulation assay (Ahmed et al 2004). The anti-obesity and anti-diabetic activity of SWR-0342SA in mice has also been reported, which reveals it to be a functionally β_3-adrenoceptor selective compound (Kiso et al 1999).

In this study, we evaluated some newly synthesized compounds – SWR-0065HA, SWR-0098NA, SWR-0315NA, SWR-0338SA, SWR-0342SA and SWR-0345HA – for their affinity for β_1-, β_2- and β_3-adrenoceptors and α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors.

Materials and Methods

Materials

The SWR-compounds used in this study were synthesized in the laboratory of Sawai Pharmaceuticals Co. Ltd (Osaka, Japan). (−)-Isoproterenol hydrochloride and Dulbecco's Modified Eagle's Medium were purchased from Sigma-Aldrich (Irvine, Ayrshire, UK). Fetal bovine serum was purchased from Sigma-Aldrich (St Louis, MO). α-Minimum essential medium was purchased from Invitrogen (NY). [^{125}I]Iodocyanopindolol ([^{125}I]ICYP) (1.85 TBq mmol^{-1}) and [^{3}H]prazosin (2856.4 GBq mmol^{-1}) were purchased from New England Nuclear Corp. (Tokyo, Japan). All other chemicals used were of the highest purity available.

Cell culture

COS-7 cells were cultured in Dulbecco's Modified Eagle's Medium supplemented with 5% fetal bovine serum and gentamicin (10 μg mL^{-1}) at 5% CO_2 and 37°C in a humidified atmosphere. When 90% confluent, the cells were transiently transfected with β_1-, β_2- and β_3-adrenoceptor by the method of Sato et al (1996). Briefly, human β-adrenoceptor subtypes in pEF-BOS were transfected into COS-7 cells using the DEAE-dextran method (Cullen 1987). The DNA sequences encoding the human β-adrenoceptor subtypes were amplified from the genomic DNA prepared from HeLa cells. Any error in the sequences was determined by the polymerase chain reaction (PCR) and was corrected thereafter. The resulting constructs were inserted into the mammalian expression vector pEF-BOS (Mizushima & Nagata 1990). The transfected cells were grown as monolayers in 100-mm dishes containing Dulbecco's Modified Eagle's Medium supplemented with 5% fetal bovine serum and gentamicin (10 μg mL^{-1}) under an atmosphere of 95% air and 5% CO_2 at 37°C.

For the cloned α_1-adrenoceptors, Chinese hamster ovary (CHO) cells were cultured in α-minimum essential medium supplemented with 10% fetal bovine serum and 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin at 5% CO_2 and 37°C in a humidified atmosphere. After transfection with the cDNA clones of human α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors, CHO cells stably expressing the human α_{1a}-, α_{1b}- and α_{1d}-adrenoceptor subtypes were grown in α-minimum essential medium supplemented with 10% fetal bovine serum and selection medium 200 μg mL^{-1} G418 at 37°C in a humidified atmosphere of 5% CO_2–95%O_2.

Membrane preparations

Forty-eight hours after the transfection, the COS-7 cells were rinsed with 10 mL of ice-cold phosphate-buffered saline (PBS) and mechanically detached by ultrasonication

in 5 mL of lysis buffer containing 5 mM Tris-HCl (pH 7.4), 2 mM EDTA, 5 μg mL^{-1} soybean trypsin inhibitor, 5 μg mL^{-1} leupeptin and 10 μg mL^{-1} benzamidine. The COS-7 cells were homogenized using a glass homogenizer and centrifuged at 45 000 *g* for 30 min at 4°C. The resultant membrane fractions were resuspended in lysis buffer and frozen at −80°C until used.

α_{1a}-, α_{1b}- and α_{1d}-adrenoceptor CHO cells were harvested by suspending the cells in ice-cold assay buffer (Tris-HCl 50 mM, EDTA 1 mM, pH 7.4), sonicating and then centrifuging at 3000 *g* for 10 min at 4°C. The supernatant was further centrifuged at 80 000 *g* for 30 min at 4°C and the resulting pellets were resuspended in the assay buffer and used for binding experiments (Israilova et al 2002).

Radioligand-binding assay

COS-7 cells were chosen for the binding experiment because of the high receptor expression levels commonly achieved in this system (Samama et al 1993). Radioligand-binding studies were carried out in assay buffer containing (in mM) 75 Tris-HCl (pH 7.4), 12.5 $MgCl_2$ and 2 EDTA at 37°C for 60 min using 5–10 μg of membrane protein. The total reaction volume was 250 μL. For saturation isotherms, membranes were incubated with varying concentrations (5–250 pM) of [^{125}I]ICYP in the absence (total binding) or presence (nonspecific binding) of 1 μM (±)-propranolol. Competition binding studies were carried out using 100 pM [^{125}I]ICYP and varying concentrations of unlabelled ligands. The reactions were stopped by dilution with 4 mL cold wash buffer containing 25 mM Tris (pH 7.5) and 1 mM $MgCl_2$ and rapid filtration over Whatman GF/C glass fibre filters. The filters were washed with an additional ice-cold wash buffer (4 mL). The radioactivity remaining on the filter was counted by a gamma counter.

For α_1-adrenoceptor saturation binding experiments, the membranes were incubated with various concentrations (10 pM to 2 nM) of [^{3}H]prazosin for 45 min at 30°C. The total incubation volume for [^{3}H]prazosin was 500 μL (α_{1a}-adrenoceptor 5 ~ 10 μg, α_{1b}-adrenoceptor 5 ~ 10 μg, α_{1d}-adrenoceptor 50 ~ 100 μg protein/tube). Nonspecific binding was defined as binding in the presence of 1 μM tamsulosin for [^{3}H]prazosin.

In competition binding experiments, membranes were incubated with 200 pM [^{3}H]prazosin and unlabelled ligand for 45 min at 30°C. Specific binding of [^{3}H]prazosin was approximately 91%, 96% and 94% of the total binding for cloned α_{1a}-, α_{1b}- and α_{1d}-adrenoceptor cells, respectively. Reactions were terminated by rapid filtration under vacuum onto Whatman GF/C filters presoaked in 0.3% polyethyleneimine for > 30 min. The filters were then washed three times with 6 mL of ice-cold 50 mM Tris-HCl (pH 7.4) and mixed in a vial with 2 mL scintillation fluid. The filter bound radioactivity was determined by liquid scintillation counting. Experiments were conducted in duplicate with at least 3 replicates examined per experiment. Binding affinities of [^{125}I]ICYP and [^{3}H]prazosin were expressed as K_d and unlabelled drugs were expressed as negative logarithm of the equilibrium dissociation constant, pK_i.

Protein assay

The protein content of the β-adrenoceptor COS-7 and α_1-adrenoceptor CHO cell membranes was measured by the methods of Lowry et al (1951) and Bradford (1976), respectively, using bovine serum albumin as the standard.

Data analysis

Binding data were analysed by the nonlinear regression and Scatchard analysis on the computer and thus dissociation constant (K_d) between receptor and antagonist and the receptor density (B_{max}) could be obtained. For β-adrenoceptor subtypes, the inhibition concentration (IC50) in displacement analysis was determined as the concentrations of ligand that inhibited [^{125}I]ICYP binding by 50%, and for α_1-adrenoceptor subtypes, the inhibition concentration (IC50) in displacement analysis was determined as the concentration of ligand that inhibited [^{3}H]prazosin binding by 50%; the values of inhibition constants (K_i) were calculated by the equation of Cheng & Prusoff (1973) and expressed as pK_i ($-\log K_i$). The results of experiments are expressed as means ± s.e.m. Graphs have been prepared with the help of computer software (GraphPad Prism version 3.00 for Windows; GraphPad Software, San Diego, CA). To compare mean values between two groups, Student's *t*-test was performed and analysis of variance was used for comparison among three and more groups to assess the significance of the difference. $P < 0.05$ was taken as significant.

Results

The binding affinity of some novel compounds, SWR-0065HA, SWR-0098NA, SWR-0315NA, SWR-0338SA, SWR-0342SA and SWR-0345HA, to recombinant human β_1-, β_2- and β_3-adrenoceptor subtypes was compared. These compounds were also investigated for any affinity for recombinant human α_{1a}-, α_{1b}- and α_{1d}-adrenoceptor subtypes. The structures of these compounds are given in Figure 1.

The membrane fractions of recombinant human β-adrenoceptor subtype cloned COS-7 cells were prepared and saturation binding experiments were performed using increasing concentrations of [^{125}I]ICYP. Saturation binding was observed in membranes from transfected COS-7 cells; a linear Scatchard transformation indicated one receptor population. Table 1 shows the calculated K_d and B_{max} values for all the β-adrenoceptor subtypes.

To investigate the binding properties of commonly used β-adrenoceptor agonists, isoproterenol and novel SWR-compounds at homogeneous populations of recombinant human β-adrenoceptor subtype cloned cells, competition experiments with [^{125}I]ICYP (100 pM) and the respective β-adrenoceptor ligands were performed. All competition curves fitted into a one-site model with the Hill slopes near unity. Figures 2A–C illustrate the displacement of [^{125}I]ICYP binding from recombinant human β-adrenoceptor subtypes by isoproterenol, SWR-0065HA,

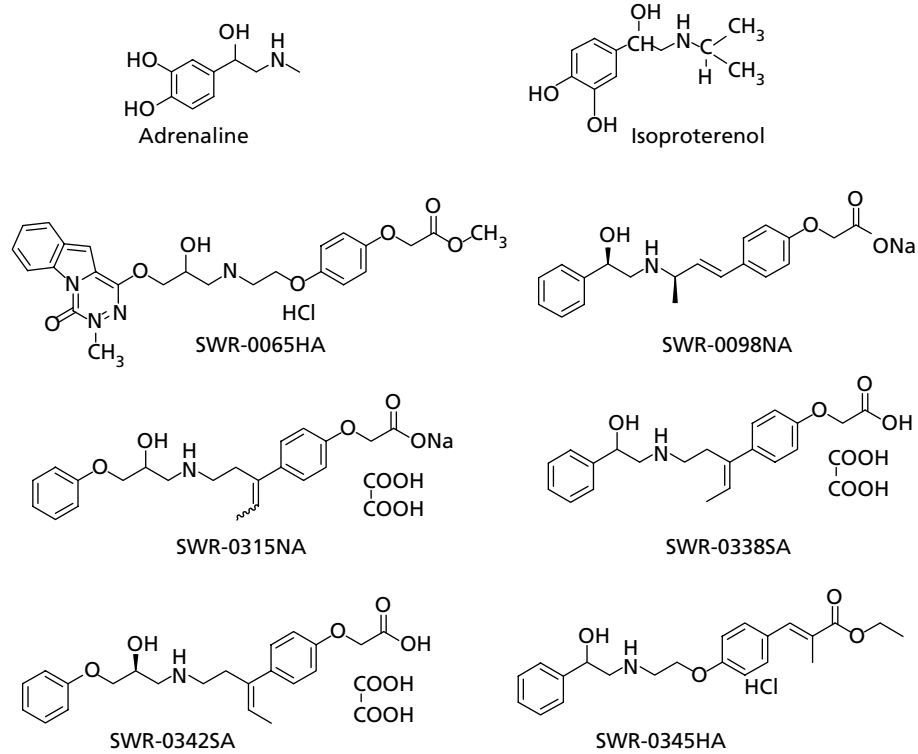

Figure 1 Structures of adrenaline, isoproterenol and the newly synthesized SWR-compounds.

Table 1 Binding properties of [^{125}I]iodocyanopindolol to membranes from COS-7 cells that express the human β-adrenoceptor subtypes

	β_1-Adrenoceptor	β_2-Adrenoceptor	β_3-Adrenoceptor
K_d (pM)	34.92 ± 2.03	18.95 ± 1.36	350.5 ± 262.07
B_{max} (fmol (mg protein)$^{-1}$)	1732 ± 43.45	666.9 ± 14.72	217.7 ± 114.6

Values indicated are means $\pm$ s.e.m. of 5 or 6 experiments.

SWR-0098NA, SWR-0315NA, SWR-0338SA, SWR-0342SA and SWR-0345HA. The pK_i values of these compounds are given in Table 2.

Compounds SWR-0315NA and SWR-0342SA showed higher binding affinity for all the β-adrenoceptor subtypes (pK_i in the range 6.17–7.18). Compound SWR-0338SA showed 13-fold and 7-fold binding selectivity for β_3-adrenoceptors against β_1- and β_2-adrenoceptors, respectively. SWR-0345HA also showed high binding selectivity (about 15-fold and 7-fold) for β_3-adrenoceptors against β_1- and β_2-adrenoceptors. Moreover, SWR-0338SA and SWR-0345HA have high functional efficacy as assessed by cAMP accumulation assay in the cultured CHO cells expressing recombinant human β_3-adrenoceptors (Ahmed et al 2003). Relative to (−)-isoproterenol, SWR-0338SA and SWR-0345HA have respective $\%E_{max}$ values of 76.95% and 83.47% for cAMP accumulation in β_3-adrenoceptor CHO cells. Although SWR-0342SA and SWR-0315NA did not show any binding selectivity towards β-adrenoceptor subtypes, they showed functional selectivity for β_3-adrenoceptors (Kiso et al 1999; Ahmed et al 2004). SWR-0098NA had a comparatively low binding profile for β-adrenoceptor subtypes, whereas SWR-0065HA had high binding affinity for β_2-adrenoceptors only, having a 3-fold and 6-fold higher binding affinity than for β_1- and β_3-adrenoceptor subtypes, respectively.

On the other hand, SWR-0098NA, SWR-0315NA and SWR-0342SA showed a similar binding affinity to α_1-adrenoceptor subtypes as adrenaline. SWR-0065HA, SWR-0315NA, SWR-0338SA, SWR-0342SA and SWR-0345HA have a 1- to 367-fold lower affinity to all α_1-adrenoceptor subtypes than to β-adrenoceptor subtypes, the only exception being SWR-0098NA, which has 2- to 22-fold higher affinity for α_1-adrenoceptor subtypes. Table 3 shows the calculated K_d and B_{max} values for all the α_1-adrenoceptor subtypes and Figures 3A–C illustrate the displacement of [^{3}H]prazosin binding from recombinant human α_1-adrenoceptor subtypes by SWR-0065HA, SWR-0098NA, SWR-0315NA, SWR-0338SA, SWR-0342SA and SWR-0345HA. All the competitive inhibition curves followed one-site binding with Hill slopes near unity.

Discussion

The radioligand binding profile of SWR compounds show that these compounds bind to human recombinant β-adrenoceptor subtypes with much greater affinity

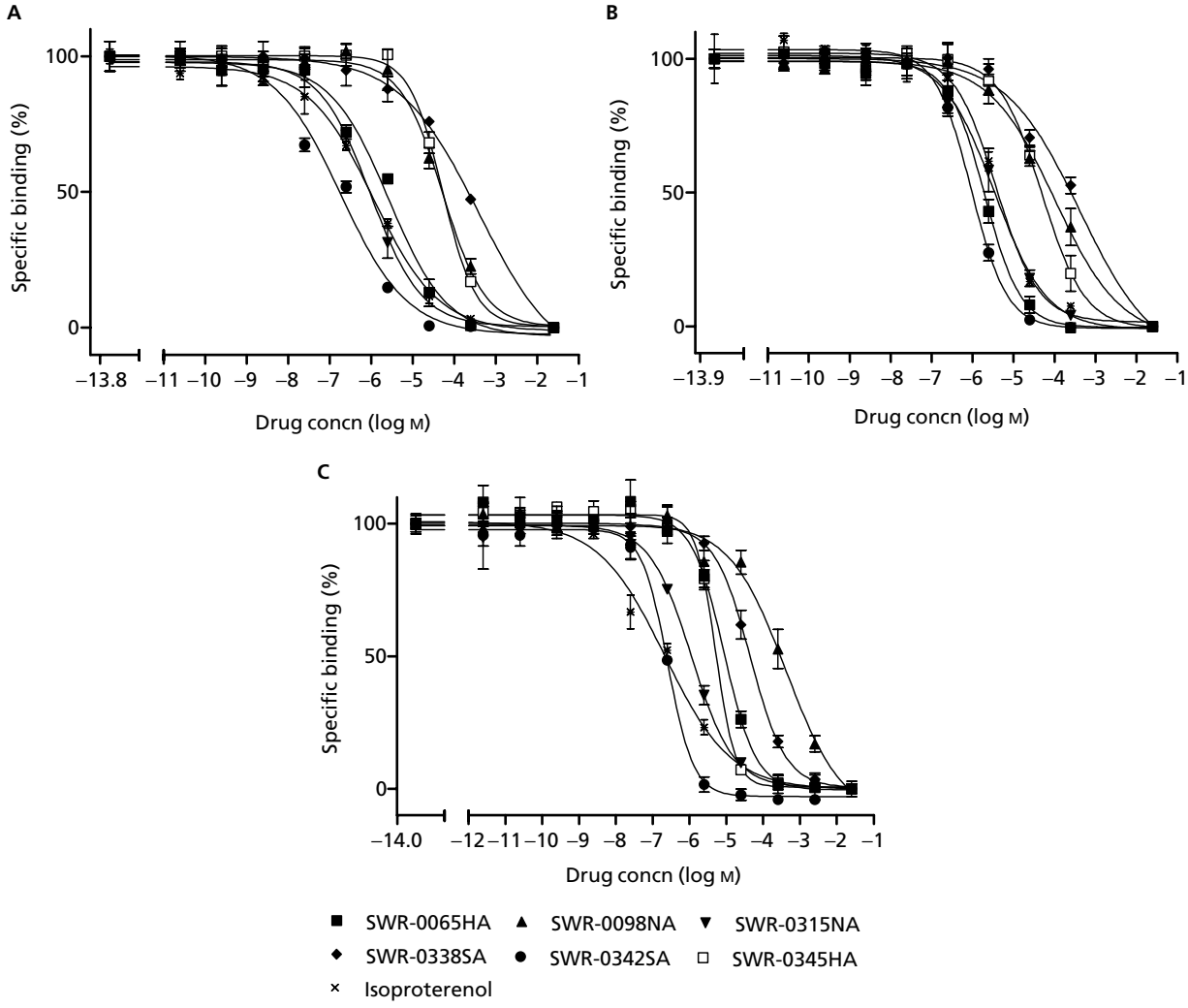

Figure 2 Competition of specific [^{125}I]ICYP binding by isoproterenol and compounds SWR-0065HA, SWR-0098NA, SWR-0315NA, SWR-0338SA, SWR-0342SA and SWR-0345HA at human recombinant β_1-, β_2- and β_3-adrenoceptor COS-7 cells (A, B and C, respectively). Data are shown as the mean ± s.e.m. of 3–5 experiments.

Table 2 Binding affinity of adrenaline and SWR compounds to human α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors expressed in CHO cells and of isoproterenol and SWR compounds to human β_1-, β_2- and β_3-adrenoceptors expressed in COS-7 cells as calculated from Figures 2 and 3

Compound	pK_i					
	α_{1a}	α_{1b}	α_{1d}	β_1	β_2	β_3
(−)-Isoproterenol				6.35 ± 0.35	6.13 ± 0.24	7.26 ± 0.33
Adrenaline[a]	6.60	5.50	5.00			
SWR-0065HA	4.78 ± 0.16	4.52 ± 0.03	4.27 ± 0.13	5.98 ± 0.04	6.42 ± 0.25	5.65 ± 0.33
SWR-0098NA	5.11 ± 0.87	5.32 ± 0.86	5.20 ± 0.92	4.69 ± 0.23	4.70 ± 0.05	3.97 ± 0.37
SWR-0315SA	5.02 ± 0.25	5.08 ± 0.01	4.77 ± 0.07	6.42 ± 0.31	6.17 ± 0.14	6.53 ± 0.40
SWR-0338SA	3.10 ± 0.36	2.34 ± 0.67	2.99 ± 0.02	3.82 ± 0.15	4.09 ± 0.39	4.94 ± 0.46
SWR-0342SA	5.19 ± 0.26	5.55 ± 0.04	5.29 ± 0.08	7.14 ± 0.03	6.74 ± 0.04	7.18 ± 0.57
SWR-0345HA	4.83 ± 0.24	4.30 ± 0.13	4.09 ± 0.11	4.69 ± 0.02	5.02 ± 0.19	5.88 ± 0.66

pK_i = negative logarithm of the equilibrium dissociation constant. Values indicated are means ± s.e.m. of 3–5 experiments. [a]Values obtained from the literature by Schwinn et al 1995.

Table 3 Binding properties of [^{3}H]prazosin to membranes from CHO cells that express the human α_1-adrenoceptor subtypes

	α_{1a}-Adrenoceptor	α_{1b}-Adrenoceptor	α_{1d}-Adrenoceptor
K_d (pM)	399.76 ± 43.38	99.04 ± 12.15	228.8 ± 37.43
B_{max} (fmol (mg protein)$^{-1}$)	2322 ± 284.13	3220.5 ± 167.8	565.52 ± 15.96

Values indicated are means ± s.e.m. of 4 or 5 experiments.

($P < 0.05$) than to α_1-adrenoceptor subtypes. Binding of SWR compounds to recombinant human β-adrenoceptor subtypes suggests that, apart from SWR-0338SA and SWR-0345HA, none of the compounds have significant selectivity in binding to β-adrenoceptor subtypes. Despite low binding affinity to β_3-adrenoceptors, SWR-0338SA and SWR-0345HA could be useful in the study of β_3-adrenoceptors in tissue. Selective ligands are helpful to study receptor-mediated functional activity.

Most of the SWR compounds showed less affinity for α_1-adrenoceptor subtypes. Compounds other than SWR-0098NA showed lower selectivity for α_1-adrenoceptor subtypes against β-adrenoceptor subtypes. SWR-0098NA has 17- to 22-fold greater affinity for α_1-adrenoceptor subtypes against β_3-adrenoceptors, and 2- to 4-fold against β_1- and β_2-adrenoceptors. SWR-0098NA, SWR-0315NA and SWR-0342SA have almost similar binding affinities towards α_1-adrenoceptor subtypes to that of adrenaline. However, SWR-0315NA and SWR-0342SA can be used in the study of β-adrenoceptors in tissues as they bind with higher selectivity for β-adrenoceptors against α_1-adrenoceptors.

Of the six compounds, SWR-0098NA, SWR-0338SA and SWR-0345HA represent the phenylethanolamine structure and SWR-0065HA, SWR-0315NA and SWR-0342SA represent the phenoxypropanolamine structure (Figure 1). β-Adrenoceptor agonists contain both the structures but α_1-adrenoceptor agonists differ in containing the phenoxypropanolamine structure. In this study, we observed that bulky substituents on the amine nitrogen of the phenoxypropanolamine structure could also render the compound a ligand for α_1-adrenoceptors. Therefore, SWR-0315NA and SWR-0342SA have almost similar affinity for α_1-adrenoceptors as adrenaline.

β-Phenylethylamine can be viewed as the parent compound of the sympathomimetic amines, consisting of a benzene ring and an ethylamine side chain. The structure permits substitutions to be made on the aromatic ring, the α- and β-carbon atoms, and the terminal amino group to yield a variety of compounds with sympathomimetic activity (Hoffman & Lefkowitz 1996).

SWR-0098NA has a phenylethanolamine structure that resembles endogenous α_1-adrenoceptor agonist structures (Figure 1). The effects of amino substitution are most readily seen in the actions of catecholamines on α-and β-receptors. An increase in the size of the alkyl substituent increases β-receptor activity (e.g., isoproterenol).

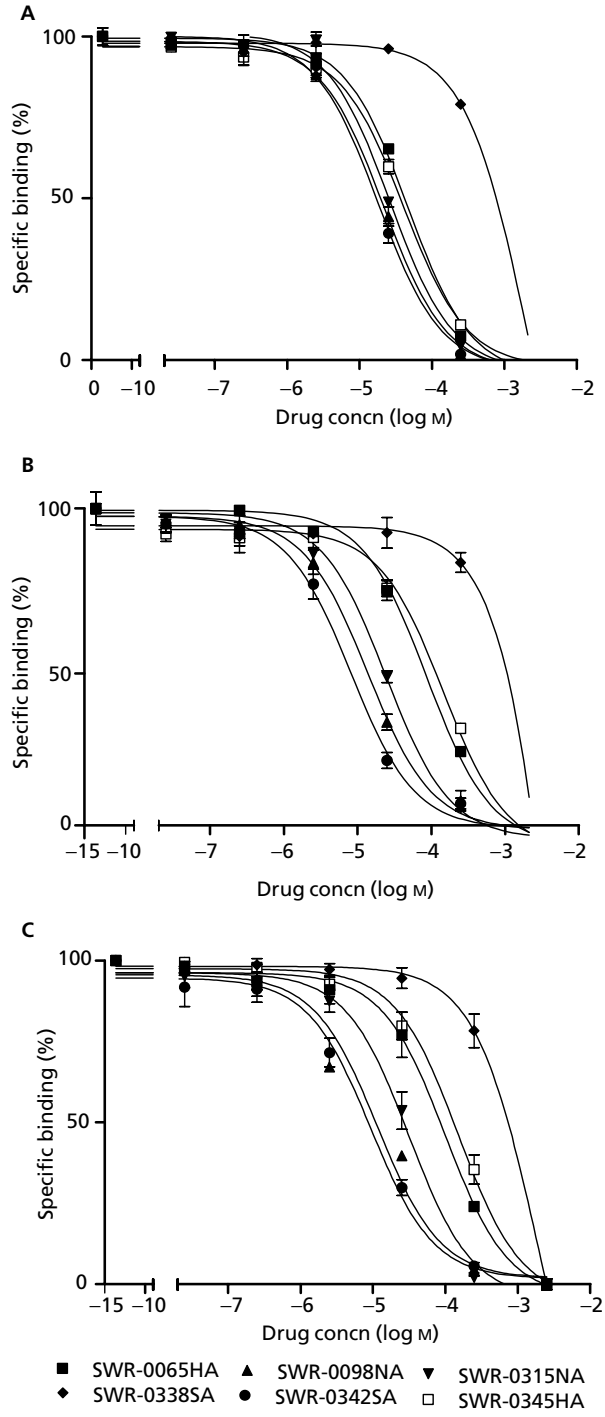

Figure 3 Competition of specific [^{3}H]prazosin binding by compounds SWR-0065HA, SWR-0098NA, SWR-0315NA, SWR-0338SA, SWR-0342SA and SWR-0345HA at human recombinant α_{1a}-, α_{1b}- and α_{1d}-adrenoceptor CHO cells (A, B and C, respectively). Data are shown as the mean ± s.e.m. of 3–5 experiments.

A notable exception is phenylephrine, which has an N-methyl substituent but is an α-selective agonist. Among these SWR compounds being studied, SWR-0098NA, SWR-0315NA and SWR-0342SA have comparatively

less substitution at the amine group, which might account for their high affinity for α_1-adrenoceptor subtypes. To evaluate their agonistic activity, the functional activity of the compounds to β-adrenoceptor and α_1-adrenoceptor subtypes has to be performed.

Conclusion

In this study we have shown that compounds SWR-0315NA and SWR-0342SA are potential ligands for recombinant human β-adrenoceptor by radioligand-binding assay. These two compounds and SWR-0098NA are potential ligands to recombinant human α_1-adrenoceptor subtypes similar to adrenaline. Moreover, SWR-0098NA has 2–22 fold greater affinity for α_1-adrenoceptor subtypes than β-adrenoceptors. It paves the way for use of SWR-0098NA in α_1-adrenoceptor studies in native tissues as well as in cloned receptors. We have also found that phenoxypropanolamine derivatives SWR-0315NA and SWR-0342SA could also be used as α_1-adrenoceptor ligands. Further investigation on their signal transduction properties will help defining their roles in β- and α_1-adrenoceptor studies.

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