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European Journal of Pharmacology 327 (1997) 79–86

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[^{3}H]*R*-Terazosin binds selectively to α_1-adrenoceptors over α_2-adrenoceptors − comparison with racemic [^{3}H]terazosin and [^{3}H]prazosin

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Received 11 November 1996; revised 10 February 1997; accepted 14 March 1997

Abstract

Most tissue sources for adrenoceptors contain a mixed population of α_1- and/or α_2-adrenoceptor subtypes; thus studies using non-specific radioligands are complicated by receptor heterogeneity. The examination of α_1-adrenoceptor radioligand binding by radiolabeled terazosin and its enantiomers was simplified by using mouse fibroblast cells, which are thymidine kinase mutant (LTK$^-$), transfected with cloned α_{1a}-, α_{1b}-, α_{1d}-adrenoceptor subtypes. [^{3}H]Terazosin and its enantiomers were equipotent at the α_{1b}-adrenoceptor. [^{3}H]*R*-Terazosin was significantly less potent than [^{3}H]terazosin and [^{3}H]*S*-terazosin at the α_{1a}- and the α_{1d}-adrenoceptors. Using tissue derived α-adrenoceptors prepared in cold 25 mM glycyl-glycine buffer, [^{3}H]prazosin, [^{3}H]terazosin and [^{3}H]*S*-terazosin bound to two sites in the rat neonatal lung preparation consistent with the presence of both α_1- and α_{2B}-adrenoceptors. The relative binding potencies of these radioligands at these two sites correlated with low affinity binding to the α_{2B}-adrenoceptor and high affinity binding to an α_1-adrenoceptor. [^{3}H]*R*-Terazosin, on the other hand, bound to a single site in the rat neonatal lung membrane preparation, most likely an α_1-adrenoceptor. Thus, [^{3}H]*R*-terazosin may be useful as a selective α_1-adrenoceptor radioligand for establishing the functional role of adrenoceptors in tissues expressing multiple subtypes.

Keywords: α_1-Adrenoceptor; Terazosin; Prazosin; Radioligand assay

1. Introduction

α-Adrenoceptors have historically been divided into α_1 and α_2 subtypes based on differences in affinity of selective drugs, in second-messenger responses and in amino-acid sequences (Bylund, 1988; Bylund et al., 1994). Tissue-derived α_1-adrenoceptors could be subdivided into α_{1A} and α_{1B} subtypes based on radioligand binding studies using [^{3}H]prazosin, chloroethylclonidine, 5-methylurapidil and WB-4101 (Morrow et al., 1985; Han et al., 1987; Gross et al., 1988). Receptor cloning studies have suggested that three separate cDNA expression products, when expressed in transformed kidney cells from the African green monkey (Cold Spring Harbor; COS-7), resembled α_{1A} (Schwinn et al., 1990), α_{1B} (Cotecchia et al., 1988), and recently designated α_{1D} (Lomasney et al., 1991; Perez et al., 1991) subtypes. Similarly, α_2-adrenoceptors have been subdivided into α_{2A}, α_{2B} and α_{2C} based on the ability of prazosin, WB4101, ARC239 and other compounds to inhibit the binding of [^{3}H]rauwolscine or [^{3}H]yohimbine to tissue homogenates (Bylund, 1985; Petrash and Bylund, 1986; Murphy and Bylund, 1988). Cloned α_{2a}-, α_{2b}- and α_{2c}-adrenoceptors have been used to support the hypothesis of subtype heterogeneity (Kobilka et al., 1987; Regan et al., 1988; Lomasney et al., 1990).

Terazosin, 2-4-(tetrahydro-2-furanyl) carbamyl 1-piperazinyl-6,7-dimethoxy-4-quinazolinamine monohydrochloride dihydrate, is an α_1-adrenoceptor antagonist structurally similar to prazosin (Luther, 1989). The saturated furan ring of terazosin markedly increases its water solubility relative to prazosin (Wilde et al., 1993). Unlike prazosin, terazosin contains a chiral center in the tetrahydrofuran residue which allows two distinct enantiomers ($S(-)$ and $R(+)$) to exist. The potencies of terazosin and

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PII S0014-2999(97)00055-1

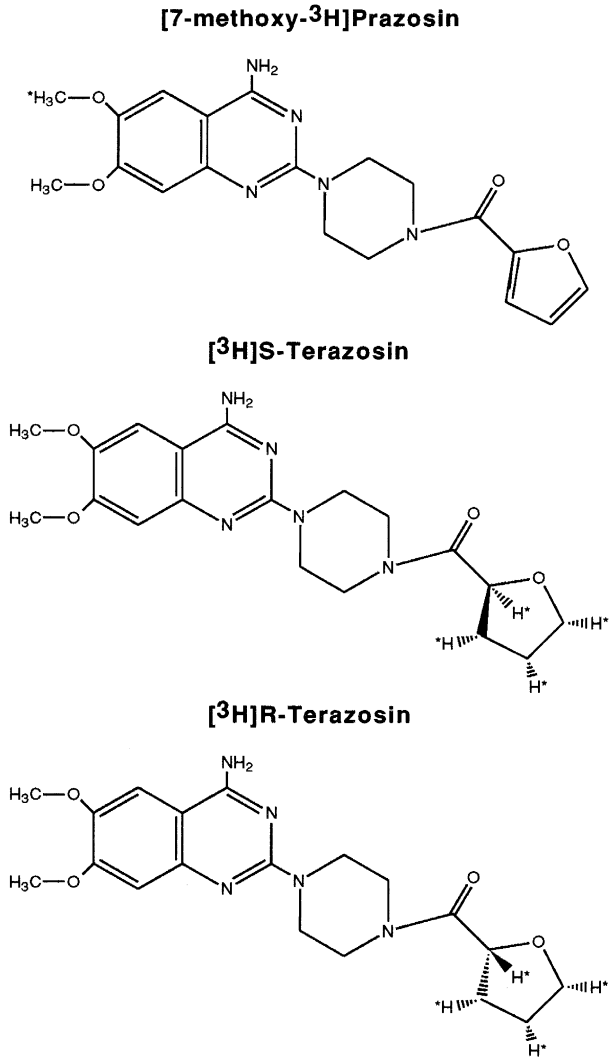


Fig. 1. The chemical structures of [7-methoxy-^{3}H]prazosin, [^{3}H]*S*-terazosin and [^{3}H]*R*-terazosin. Asterisks (*) indicate sites of tritiation.

its enantiomers were compared using bovine prostate, rat brain, rat heart, rat liver and canine aorta tissue preparations in radioligand binding assays (Meretyk et al., 1992; Maruyama et al., 1994; Kyncl et al., 1990). Interpretation of the potencies of unlabeled terazosin and its enantiomers was problematic in tissue membrane preparations containing a heterogeneous population of α_1-adrenoceptors. The pharmacologically defined α_{1A}-, α_{1B}-, α_{1D}-adrenoceptors have been cloned and stably expressed in cells lines thus providing models of each subtype to study without the complication of mixed receptor populations. Both enantiomers of terazosin had similar potencies at the cloned α_1-adrenoceptor subtypes as the racemate, which were approximately 10-fold less potent than prazosin, as shown in both receptor binding and functional bioassays (Hancock et al., 1995b).

Prazosin has been used to study the heterogeneity of α_2-adrenoceptors because it can discriminate between the subtypes (Bylund, 1985; Hancock et al., 1995a; Latifpour and Bylund, 1983). Prazosin was 10-fold more potent at the α_{2B}-adrenoceptor than at the α_{2c}-adrenoceptor and 10-fold more potent at the α_{2c}-adrenoceptor than the α_{2a}-adrenoceptor (Hancock et al., 1995b). Racemic terazosin and the *S*-enantiomer had similar potencies at the subtypes of the α_2-adrenoceptor as prazosin (Hancock et al., 1995b). However, the *R*-enantiomer was significantly less potent than prazosin at these receptor subtypes in both receptor binding and functional studies (Hancock et al., 1995b). Also, the *R*-enantiomer was significantly less potent at the α_{2a}-adrenoceptor than at the α_{2B}-adrenoceptor and the α_{2c}-adrenoceptor (Hancock et al., 1995b). These results suggest *R*-terazosin may be utilized to differentiate functional responses mediated by subtypes of α_2-adrenoceptors. Because of the binding characteristics of the *R*-enantiomer of terazosin, we thought that [^{3}H]*R*-terazosin (Fig. 1) would be useful as a tool to study receptor binding in mixed α_1- and α_2-adrenoceptor populations. We have tested this hypothesis using the rat neonatal lung, which contains both α_1- and α_2-adrenoceptors (Latifpour and Bylund, 1983), as a model system and have characterized the binding of [^{3}H]terazosin, [^{3}H]*S*-terazosin and [^{3}H]*R*-terazosin to α_1-adrenoceptors.

2. Materials and methods

[^{3}H]Terazosin, synthesized by Dupont/NEN by the catalytic reduction of prazosin in solution at one atmosphere with tritium gas using Pd/C as the catalyst, was isolated by filtration, evaporated to dryness in vacuo, and purified by preparative HPLC. Samples were injected using a Rheodyne Model 7125 syringe-loading sample injector with a 200 μl loop. The chromatography mobile phases were delivered by a Perkin-Elmer Model 410 quaternary pump. Peaks were detected with an Applied Biosystems Model 785A UV detector set at 246 nm which was connected in series to a Packard Radiomatic Flo-One Beta Model 500 radioactivity flow monitor.

Purification of [^{3}H]*R*-terazosin and [^{3}H]*S*-terazosin from racemic [^{3}H]terazosin and determination of the optical purities were performed using a Chiralpak AD (250 × 4.6 mm I.D.) column (Chiral Technologies, Exton, PA, USA) and *n*-hexane/ethanol (75:25) mobile phase at a flow-rate of 1.5 ml/min. Radiochemical purity determination was performed using an Alltima ODS, 5 micron (250 × 4.6 I.D.) column (Alltech, Deerfield, IL, USA) with a mobile phase consisting of potassium phosphate, monobasic, with pH adjusted to 3.2 with phosphoric acid/acetonitrile (80:20) and set at a flow-rate of 1.0 ml/min. Cocktail (Packard Flo-Scint III) was pumped at 3.0 ml/min. From 22.0 mCi of racemic [^{3}H]terazosin (specific activity ≈ 36.6 Ci/mmol), 7.83 mCi were recovered of the *R*-enantiomer with > 99% and 7.87 mCi of the *S*-enantiomer with > 97% radiochemical/optical purity. A

portion (3.5 mCi) of the *S*-enantiomer was repurified to give 2.25 mCi of > 99% radiochemical/optical purity.

For receptor characterization studies, cell membranes from LTK$^-$ cells stably expressing cloned human α_{1a}-, hamster α_{1b}-, or rat α_{1d}-adrenoceptor subtypes and from rat neonatal lungs were prepared and stored at −70°C as described previously (α_1-adrenoceptors: Knepper et al., 1995; Vodenlich et al., 1993; Hancock et al., 1995a; rat neonatal lungs: Latifpour et al., 1982; Hancock et al., 1995c). For saturation experiments, serial concentrations of [^{3}H]terazosin, [^{3}H]*R*-terazosin, [^{3}H]*S*-terazosin (0.07–7.0 nM, 450 μl), or [^{3}H]prazosin (75–80 Ci/mmol, Dupont-NEN Corp.; 0.01–1.0 nM, 450 μl) prepared in ice-cold 25 mM glycyl-glycine buffer (pH = 7.4 at 25°C) were added to polystyrene tubes containing either 50 μl of H_2O (total binding) or 50 μl of phentolamine (final concentration = 10 μM, non-specific binding). Frozen receptor cell membrane preparations were thawed, diluted 10-fold with ice-cold 25 mM glycyl-glycine buffer and samples of the membrane preparation (200 μl) were withdrawn for determination of protein concentration. The cloned α_1-adrenoceptor membrane preparation was then diluted (8-fold) an additional time as compared to the rat neonatal lung preparation and kept on ice. A 500 μl aliquot of the membrane preparation (0.834 mg wet weight/tube for α_1-adrenoceptors and 6.66 mg wet weight/tube for rat neonatal lung) was added to each tube and incubated for 60 min at 25°C for α_1-adrenoceptors, or 120 min at 0–4°C (Perry and U'Prichard, 1981) for the rat neonatal lung membrane preparation. Radioligand binding was terminated by filtering through Whatman GF/B filters followed by 4 rinses with 50 mM Tris-HCl buffer. The amount of bound radiolabel was determined by liquid scintillation counting.

Competition assays for α_{1b}-adrenoceptors employing [^{3}H]prazosin, [^{3}H]terazosin, [^{3}H]*R*-terazosin and [^{3}H]*S*-terazosin were performed as described previously (Knepper et al., 1995; Greengrass and Bremner, 1979) with the exception that the membrane preparation and radioligands were diluted with 25 mM glycyl-glycine buffer instead of 50 mM Tris-HCl. The following unlabeled compounds were used in competition assays with the adrenoceptor radiolabels: prazosin, terazosin, *S*-terazosin, *R*-terazosin, ARC239 (2[2-4(*o*-methoxyphenyl)-piperazine-1-yl] 4,4-dimethyl-1,3(2*H*-4*H*) isoquinolindione), WB-4101 [2-(2,6-dimethoxyphenoxyethyl) aminomethyl-1,4-benzodioxane], rauwolscine, phentolamine, phenylephrine, norepinephrine, A-61603 (*N*-[5-(4,5-dihydro-1*H*-imidazol-2yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1yl] methanesulfonamide · HCl), a selective α_{1A}-adrenoceptor agonist (Knepper et al., 1995), and A-53693 (2-methyl-2,3,3*a*,4,5,9*b*-hexahydro-6,7-dihydroxy-1*H*-benz(*e*)isoindole), a selective α_2-adrenoceptor agonist (Hancock et al., 1988).

For on-rate kinetics studies, 500 μl of the membrane preparation was added to each tube and incubated for various time points between 0.0–60 min at 0°C. For the off-rate kinetics, 500 μl of the membrane preparation was added to each tube and incubated for 60 min at 0°C prior to addition of 50 μl of phentolamine (final concentration = 100 μM), to initiate dissociation, at various time points. The calculation of the on-rate (k_{+1}) was determined using the pseudo-first-order method (Bylund and Yamamura, 1990). The K_d values were determined from dividing the off-rate (k_{-1}) values by the k_{+1} values.

The binding data were analyzed using the nonlinear least squares program SCAFIT (Hancock and Marsh, 1984). The data were first fit to a one-binding site and then multiple-binding site models. The multiple-binding site model was accepted if the residual sums of squares were statistically less for a multiple-binding site fit of the data than for the less complex fit, as determined by *F*-test comparison. The experimental results are expressed as the mean ± S.E.M. for pK_d, B_{max} and Hill coefficient (n_H) for each radioligand tested at each receptor. Statistical analysis was performed by analysis of variance followed by Tukey's protected *t*-test where *P* values less than 0.05 were considered significant.

3. Results

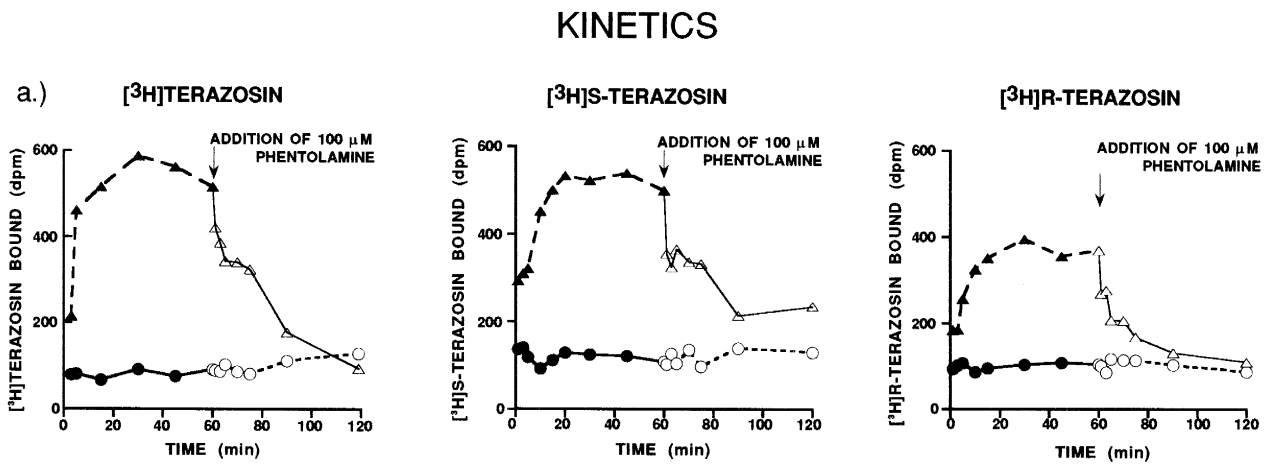
The binding of [^{3}H]prazosin, [^{3}H]terazosin, [^{3}H]*R*-terazosin and [^{3}H]*S*-terazosin at human α_{1a}-adrenoceptor, hamster α_{1b}-adrenoceptor and rat α_{1d}-adrenoceptor was saturable with relatively low non-specific binding (data not shown). In preliminary assays, several different buffers were tested in the radioligand binding assays because the typical buffer (50 mM Tris-HCl) used with [^{3}H]prazosin (Greengrass and Bremner, 1979) gave unacceptable signal-to-noise ratios for the radiolabeled terazosin and its enantiomers (data not shown). The lowest non-specific binding of [^{3}H]terazosin and its radiolabeled enantiomers was observed in the glycyl-glycine buffer which was chosen for subsequent experiments. All four radioligands bound to the same homogeneous population of receptors in the α_1-adrenoceptor clones based on B_{max} values and from best-fit analysis of the one-binding site model (Table 1). [^{3}H]Terazosin and its radiolabeled enantiomers required 10-fold higher concentrations than [^{3}H]prazosin to reach saturable binding. Hence, [^{3}H]prazosin was significantly more potent at all three α_1-adrenoceptor clones tested. [^{3}H]*R*-Terazosin was significantly less potent than [^{3}H]terazosin and [^{3}H]*S*-terazosin at the human α_{1a}-adrenoceptor and the rat α_{1d}-adrenoceptor (Table 1). However, [^{3}H]terazosin, [^{3}H]*R*-terazosin and [^{3}H]*S*-terazosin had similar pK_d values at the hamster α_{1b}-adrenoceptor. [^{3}H]Terazosin and [^{3}H]*S*-terazosin appeared to have similar potency at all three α_1-adrenoceptor clones tested (Table 1).

The binding kinetics for [^{3}H]terazosin, [^{3}H]*S*-terazosin and [^{3}H]*R*-terazosin were determined for the hamster α_{1b}-adrenoceptor (Fig. 2). All three radioligands reached

Table 1
Comparison of binding of [^{3}H]prazosin, [^{3}H]terazosin, [^{3}H]*S*-terazosin and [^{3}H]*R*-terazosin at α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors

	Radioligand	pK_d (M)	B_{max} (fmol/mg protein)	n_H
Clonal human				
α_{1a}	[^{3}H]Prazosin	10.9 ± 0.04 [a]	350 ± 39	1.01 ± 0.06
α_{1a}	[^{3}H]Terazosin	9.69 ± 0.03	306 ± 28	0.79 ± 0.07
α_{1a}	[^{3}H]*S*-Terazosin	9.83 ± 0.1	400 ± 14	0.89 ± 0.06
α_{1a}	[^{3}H]*R*-Terazosin	9.24 ± 0.1 [b]	359 ± 16	1.03 ± 0.05
Clonal hamster				
α_{1b}	[^{3}H]Prazosin	11.0 ± 0.1 [a]	460 ± 4.7	0.77 ± 0.02
α_{1b}	[^{3}H]Terazosin	10.2 ± 0.1	442 ± 50	0.86 ± 0.05
α_{1b}	[^{3}H]*S*-Terazosin	10.2 ± 0.1	414 ± 71	0.77 ± 0.07
α_{1b}	[^{3}H]*R*-Terazosin	10.1 ± 0.01	384 ± 68	0.96 ± 0.11
Clonal rat				
α_{1d}	[^{3}H]Prazosin	11.2 ± 0.1 [a]	1782 ± 190	0.95 ± 0.10
α_{1d}	[^{3}H]Terazosin	9.83 ± 0.1	1571 ± 166	0.95 ± 0.07
α_{1d}	[^{3}H]*S*-Terazosin	9.85 ± 0.1	1747 ± 170	1.05 ± 0.13
α_{1d}	[^{3}H]*R*-Terazosin	9.49 ± 0.1 [b]	1603 ± 181	0.93 ± 0.02

pK_d, B_{max} values and Hill coefficients (n_H) were derived from least squares regression analysis of Scatchard plots. Saturation assays were performed as described in Section 2. The range of concentrations of radioligand was generally 0.005–5.0 nM for [^{3}H]terazosin, [^{3}H]*S*-terazosin and [^{3}H]*R*-terazosin, and 0.001–1.0 nM for [^{3}H]prazosin. The potencies of the radiolabels have been expressed by the negative logarithms of their K_d values (pK_d). Values for pK_d are the geometric mean ± S.E.M. and for B_{max} are the mean ± S.E.M. of 3–5 experiments.
[a] [^{3}H]Prazosin significantly different from [^{3}H]terazosin, [^{3}H]*S*-terazosin and [^{3}H]*R*-terazosin.
[b] [^{3}H]*R*-Terazosin significantly different from [^{3}H]prazosin, [^{3}H]terazosin and [^{3}H]*S*-terazosin.



b.)

RADIOLIGAND	kobs (min^{-1})	k-1 (min^{-1})	k+1 (min^{-1}M^{-1})	pKd (M)
[3H]TERAZOSIN	0.072 ± 0.007	0.019 ± 0.003	1.01 x 10^8 ± 1.46 X 10^7	9.72 ± 0.13
[3H]S-TERAZOSIN	0.173 ± 0.053	0.014 ± 0.002	2.39 x 10^8 ± 7.66 x 10^7	10.1 ± 0.18*
[3H]R-TERAZOSIN	0.076 ± 0.009	0.020 ± 0.002	7.99 x 10^7 ± 1.17 x 10^7	9.59 ± 0.05

Fig. 2. (●) NSB (on-rate); (▲) specific (on-rate). (a) Radioligand kinetics assay of [^{3}H]terazosin, [^{3}H]*S*-terazosin or [^{3}H]*R*-terazosin to the hamster α_{1b}-adrenoceptor. For the on-rate kinetics, 50 µl of H_2O or 10 µM phentolamine, 450 µl of the radioligand and 500 µl of the receptor preparation were added to each tube and incubated for 0–60 min at 0°C. Radioligand binding was terminated by filtering with Whatman GF/B filters, rinsing 4 times with 50 mM Tris-HCl buffer. (○) NSB (off-rate); (△) specific (off-rate). For the off-rate kinetics, 50 µl of H_2O or 10 µM phentolamine, 450 µl of the radioligand and 500 µl of the receptor preparation were added to each tube and incubated for 60 min at 0°C prior to addition of 50 µl of 100 µM phentolamine, to initiate dissociation at various time points. (b) k_{obs} and k_{-1} values were derived from least-squares regression analysis of pseudo-first-order association and dissociation plots. The k_{+1} and pK_d values expressed as mean ± S.E.M. were calculated from $k_{+1} = k_{obs} - (k_{-1})$/radioligand concentration and from $k_d = k_{-1}/k_{+1}$, respectively. * The mean pK_d value of [^{3}H]*S*-terazosin was significantly different ($P < 0.05$) from [^{3}H]terazosin and [^{3}H]*R*-terazosin ($n = 3$–5 experiments).

Table 2
Inhibition of [^{3}H]prazosin, [^{3}H]terazosin, [^{3}H]*S*-terazosin and [^{3}H]*R*-terazosin binding to the α_{1b}-adrenoceptor by various adrenergic antagonists and agonists

Compounds	[^{3}H]Prazosin pK_i (M)	[^{3}H]Terazosin pK_i (M)	[^{3}H]*S*-Terazosin pK_i (M)	[^{3}H]*R*-Terazosin pK_i (M)
Prazosin	10.5 ± 0.06	11.0 ± 0.12	11.0 ± 0.15	10.9 ± 0.17
Terazosin	9.85 ± 0.12	10.1 ± 0.17	9.93 ± 0.09	10.3 ± 0.10
S-Terazosin	9.68 ± 0.09	9.86 ± 0.11	9.71 ± 0.12	9.92 ± 0.03
R-Terazosin	9.49 ± 0.09	9.75 ± 0.12	9.68 ± 0.10	9.71 ± 0.08
ARC 239	9.64 ± 0.07	9.98 ± 0.06	9.83 ± 0.10	9.94 ± 0.05
WB-4101	9.43 ± 0.10	9.69 ± 0.23	9.59 ± 0.10	9.75 ± 0.11
Phentolamine	8.26 ± 0.08	8.67 ± 0.14	8.45 ± 0.17	8.44 ± 0.17
Rauwolscine	6.93 ± 0.07	7.18 ± 0.13	7.27 ± 0.14	7.19 ± 0.09
A-53693	6.63 ± 0.06	7.01 ± 0.12	6.80 ± 0.09	6.83 ± 0.03
Norepinephrine	6.43 ± 0.22	6.92 ± 0.18	6.54 ± 0.25	6.71 ± 0.18
A-61603	6.06 ± 0.13	6.58 ± 0.16	6.56 ± 0.27	6.42 ± 0.20
Phenylephrine	5.80 ± 0.13	6.19 ± 0.26	6.22 ± 0.18	6.18 ± 0.12

Inhibition experiments were performed by incubating an aliquot of the α_{1b}-adrenoceptor membrane preparation with [^{3}H]prazosin (0.2 nM), [^{3}H]terazosin (2.0 nM), [^{3}H]*S*-terazosin (2.0 nM), or [^{3}H]*R*-terazosin (2.0 nM) in the presence of 11 concentrations of unlabeled drug. IC_{50} values were determined and K_i values were calculated using the Cheng-Prusoff equation. The potencies of the drugs have been expressed by the negative logarithms of their K_i values (pK_i). The pK_i values are means ± S.E.M. for 3–5 experiments.

steady-state equilibrium within 20 min at 0°C with similar total specific binding. Specific binding of [^{3}H]terazosin, [^{3}H]*R*-terazosin, or [^{3}H]*S*-terazosin accounted for approximately 62%, 73% and 73% of total radioligand binding in the α_{1b}-adrenoceptor membrane preparation, respectively. The 0°C incubation temperature for these experiments improved the kinetic measurements by slowing the reaction rate allowing for more accurate measurement of the on-rate and off-rate kinetics. Attempts to measure on-rates at 25°C were hampered by the extremely rapid binding of [^{3}H]terazosin and its enantiomers, in contrast to [^{3}H]prazosin which reached half-maximal binding in ~2 min (data not shown). Upon the addition of 100 μM phentolamine to initiate dissociation, the specific binding for all three radiolabels was reduced while non-specific binding was unaffected (Fig. 2a). The observed on-rate (k_{obs}), k_{-1} and calculated k_{+1} values for all three radioligands were not significantly different; however, the kineti-

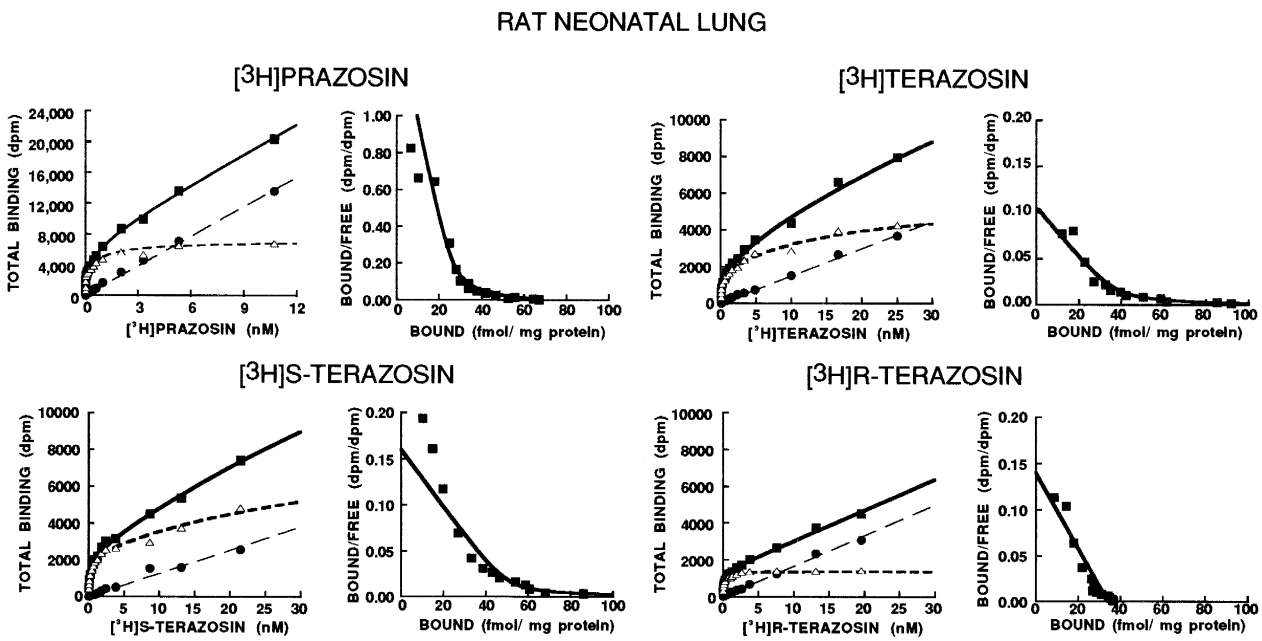


Fig. 3. (■) Total; (●) NSB; (△) specific. Radioligand binding assay of [^{3}H]prazosin, [^{3}H]terazosin, [^{3}H]*S*-terazosin or [^{3}H]*R*-terazosin to the rat neonatal lung preparation. Triplicate polystyrene tubes containing either 50 μl of H_2O or 10 μM phentolamine, 450 μl of the radioligand and 500 μl of the receptor preparation were incubated for 120 min at 0°C. Radioligand binding was terminated by filtering with Whatman GF/B filters, rinsing 4 times with 50 mM Tris-HCl buffer.

Table 3
Comparison of binding of [^{3}H]prazosin, [^{3}H]terazosin, [^{3}H]*S*-terazosin and [^{3}H]*R*-terazosin in rat neonatal lung membranes

Rat neonatal lung	Radioligand	pK_d (M)	B_{max} (fmol/mg protein)	n_H
High affinity site				
α_1	[^{3}H]Prazosin	11.1 ± 0.1 [a]	23.9 ± 1.2	ND
α_1	[^{3}H]Terazosin	10.3 ± 0.3	24.9 ± 6.0	ND
α_1	[^{3}H]*S*-Terazosin	10.6 ± 0.5	25.9 ± 13	ND
α_1	[^{3}H]*R*-Terazosin	9.9 ± 0.1 [b]	39.9 ± 4.2	0.72 ± 0.11
Low affinity site				
α_{2B}	[^{3}H]Prazosin	9.0 ± 0.02	43.8 ± 5.3	ND
α_{2B}	[^{3}H]Terazosin	8.2 ± 0.3	115 ± 38	ND
α_{2B}	[^{3}H]*S*-Terazosin	8.6 ± 0.6	101 ± 35	ND
α_{2B}	[^{3}H]*R*-Terazosin	No affinity	No affinity	ND

pK_d, B_{max} values and Hill coefficients (n_H) were derived from least squares regression analysis of Scatchard plots. Saturation assays were performed as described in Section 2. The range of concentrations of radioligand was generally 0.025–25 nM for [^{3}H]terazosin, [^{3}H]*S*-terazosin and [^{3}H]*R*-terazosin, and 0.01–10 nM for [^{3}H]prazosin. The potencies of the radiolabels have been expressed by the negative logarithms of their K_d values (pK_d). Values for pK_d are the geometric mean ± S.E.M. and for B_{max} are the mean ± S.E.M. of 3–5 experiments. ND means values not determined.
[a] [^{3}H]Prazosin significantly different from [^{3}H]terazosin and [^{3}H]*R*-terazosin.
[b] [^{3}H]*R*-Terazosin significantly different from [^{3}H]prazosin and [^{3}H]*S*-terazosin.

cally derived pK_d for [^{3}H]*S*-terazosin was significantly more potent than the pK_d derived for [^{3}H]terazosin and [^{3}H]*R*-terazosin (Fig. 2b).

Several standard α-adrenoceptor ligands were tested in competition assays against [^{3}H]prazosin, [^{3}H]terazosin, [^{3}H]*S*-terazosin, or [^{3}H]*R*-terazosin at the hamster α_{1b}-adrenoceptor. The antagonists were prazosin, terazosin, *S*-terazosin, *R*-terazosin, ARC239, WB-4101, rauwolscine and phentolamine while the agonists were phenylephrine, norepinephrine, A-61603 and A-53693. The calculated pK_i values for each ligand were similar across the four radioligands (Table 2). Unlabeled prazosin was the most potent at displacing [^{3}H]prazosin, [^{3}H]terazosin, [^{3}H]*S*-terazosin and [^{3}H]*R*-terazosin from the hamster α_{1b}-adrenoceptor. Terazosin, *S*-terazosin, ARC239, *R*-terazosin and WB-4101 followed closely in potency with pK_i values between 9.4 and 10.3. The agonists were the weakest at inhibiting [^{3}H]prazosin, [^{3}H]terazosin, [^{3}H]*S*-terazosin and [^{3}H]*R*-terazosin at the hamster α_{1b}-adrenoceptor.

The binding of [^{3}H]prazosin and [^{3}H]*R*-terazosin in rat neonatal lung preparations was saturable at concentrations ≥ 5 nM; however, the binding of [^{3}H]terazosin and [^{3}H]*S*-terazosin in this tissue did not saturate until approximately 30 nM (Fig. 3). Scatchard plots of [^{3}H]prazosin, [^{3}H]terazosin and [^{3}H]*S*-terazosin binding in the rat neonatal lung revealed multiple binding sites with curvilinear slopes consistent with a two-binding site model. In contrast, [^{3}H]*R*-terazosin appeared to bind only one site (Hill coefficient not significantly different from unity) (Table 3). The B_{max} values of the high-affinity sites for [^{3}H]prazosin, [^{3}H]terazosin and [^{3}H]*S*-terazosin, and the only binding site for [^{3}H]*R*-terazosin were similar, with K_d values consistent with those of an α_1-adrenoceptor. At the high affinity site, [^{3}H]prazosin was significantly more potent than [^{3}H]terazosin and [^{3}H]*R*-terazosin but not [^{3}H]*S*-terazosin, which was significantly more potent than its stereoisomer (Table 3). At the low affinity site, [^{3}H]prazosin, [^{3}H]terazosin and [^{3}H]*S*-terazosin have similar potencies of around 9 nM while [^{3}H]*R*-terazosin exhibited no specific binding at all (Fig. 3). The B_{max} values of the low affinity sites for [^{3}H]prazosin, [^{3}H]terazosin and [^{3}H]*S*-terazosin in the rat neonatal lung are similar to the $B_{max} = 90.2 \pm 13.6$ fmol/mg protein for the α_2-adrenoceptor selective antagonist [^{3}H]rauwolscine in this tissue (Hancock et al., 1995b). Thus, the low affinity site in the rat neonatal lung membrane preparations probably represented binding of [^{3}H]prazosin, [^{3}H]terazosin and [^{3}H]*S*-terazosin to the α_{2B}-adrenoceptor.

4. Discussion

The potencies of [^{3}H]terazosin and its radiolabeled enantiomers at the α_1-adrenoceptor subtypes were 10-fold higher than previously reported results for the unlabeled racemate and its enantiomers (Hancock et al., 1995b). [^{3}H]Prazosin binding was 5–10-times more potent in the α_1-adrenoceptor subtypes than previously reported (Hancock et al., 1995b). This effect was attributed to the use of 25 mM glycyl-glycine buffer which reduced nonspecific binding in the clonal cell membrane preparations as compared to several other buffers tested but also increased the affinity of the radioligands several-fold. Glycyl-glycine buffer had been previously shown to improve the binding of α_2-adrenoceptor antagonists (Latifpour et al., 1982) and α_1-adrenoceptor antagonists (Jones et al., 1987). All four radioligands had similar rank order potencies at the α_1-adrenoceptor subtypes as their unlabeled counterparts (Hancock et al., 1995b). [^{3}H]Terazosin and its radiolabeled enantiomers had the highest potency at the

α_{1b}-adrenoceptor, whereas [^{3}H]prazosin appeared to have similar potency across all three α_1-adrenoceptor subtypes tested. Analysis of the Scatchard plots of [^{3}H]prazosin, [^{3}H]terazosin, [^{3}H]*S*-terazosin and [^{3}H]*R*-terazosin suggests the radiolabels are binding the same receptor population for each clonal α_1-adrenoceptor membrane preparation.

The binding kinetics of [^{3}H]terazosin, [^{3}H]*S*-terazosin and [^{3}H]*R*-terazosin suggest that each radioligand binds with similar on- and off-rates. Analysis of the kinetics experiments proved to be very difficult because radiolabeled terazosin and its enantiomers bound the α_{1b}-adrenoceptors so quickly that at the earliest time point, up to half of the receptors were occupied. To decrease the on-rates, the radioligands were incubated with the cell membranes at 0°C instead of 25°C, allowing for more accurate determination of the on-rates, which were heavily influenced by the first few time points. The calculated pK_d values from the kinetics experiments for [^{3}H]terazosin, [^{3}H]*S*-terazosin and [^{3}H]*R*-terazosin were similar to those determined by the equilibrium binding experiments. However, the kinetically derived pK_d for [^{3}H]*S*-terazosin was significantly different from those for [^{3}H]terazosin and [^{3}H]*R*-terazosin. This may be the result of the inherent difficulties with these assays, since in direct saturation binding experiments the enantiomers of terazosin bound with more equal affinities.

[^{3}H]Terazosin, [^{3}H]*S*-terazosin and [^{3}H]*R*-terazosin were also compared in competition assays against several standard adrenoceptor antagonists and agonists. Across all four radioligands, each antagonist and agonist had the same rank potencies at the α_{1b}-adrenoceptor and very similar absolute affinities. Unlabeled prazosin was the most potent at displacing the radioligands. Unlabeled racemic terazosin and its unlabeled enantiomers, as well as ARC239 and WB4101 were approximately equipotent at displacing the radiolabels. The agonists were the weakest in these inhibition studies. This order of potency of the antagonists and agonists was consistent with the classical pattern characterizing α-adrenoceptors and suggests that all four radiolabels bind to the α_{1b}-adrenoceptor in a similar manner. Based on previous analysis of unlabeled terazosin and its enantiomers (Meretyk et al., 1992; Maruyama et al., 1994; Kyncl et al., 1990; Hancock et al., 1995b), it appears that [^{3}H]terazosin, [^{3}H]*S*-terazosin and [^{3}H]*R*-terazosin have the same binding characteristics as the unlabeled compounds at α_1-adrenoceptors.

Rat neonatal lung membrane contains α_1- and α_{2B}-adrenoceptors (Latifpour and Bylund, 1983). [^{3}H]Prazosin, [^{3}H]terazosin and [^{3}H]*S*-terazosin bind to both the α_{2B}-adrenoceptor as well as an α_1-adrenoceptor in rat neonatal lung membranes when a glycyl-glycine buffer is used. However, at concentrations up to 30 nM [^{3}H]*R*-terazosin bound only to the α_1-adrenoceptor population. This observation was anticipated since unlabeled *R*-terazosin had a pK_i of 6.5 M for the α_{2B}-adrenoceptor (Hancock et al., 1995b). Unlabeled *R*-terazosin was more potent at the α_1-adrenoceptors (pK_i values ranging from 8.2–9.0 M) than at the α_2-adrenoceptors (pK_i values ranging from 5.4–6.5 M) (Hancock et al., 1995b). These results for unlabeled *R*-terazosin binding at α_2-adrenoceptors together with the lack of specific binding of [^{3}H]*R*-terazosin at the α_{2B}-adrenoceptor in the rat neonatal lung provide strong evidence that [^{3}H]*R*-terazosin does not bind to α_2-adrenoceptors. In most studies, [^{3}H]prazosin binds almost exclusively to α_1-adrenoceptors, particularly if the typical Tris-based buffers are used. However, in autoradiographic studies or other assays where the presence of α_{2B}-adrenoceptors, tissue constituents, or other factors may be harder to control, the possibility exists for heterogeneous binding by [^{3}H]prazosin to additional non-α_1-adrenoceptor sites. Since [^{3}H]*R*-terazosin appears to be more selective for α_1-adrenoceptors over α_2-adrenoceptors, this radioligand or its unlabeled congener may be useful for receptor binding assays, autoradiographic analysis and associated functional analysis of α_1- and α_2-adrenoceptors.

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