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Characterization of specific binding of [^{125}I]L-762,459, a selective α_{1A}-adrenoceptor radioligand to rat and human tissues

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Abstract

L-762,459 ((±)1-(3-{[5-carbamoyl-2-{2-[(4-hydroxy-3-iodobenzimidoyl)-amino]-ethoxy-methyl}-6-methyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3-carbonyl]-amino}-propyl)-4-phenyl-1-piperidine-4-carboxylic acid methyl ester), an analog of a series of dihydropyridines previously reported to be selective α_{1A}-adrenoceptor subtype antagonists was found to have α_{1A}-adrenoceptor subtype selectivity (K_i (nM), 1a = 1.3, 1b = 240, 1d = 280). Specific [^{125}I]L-762,459 binding was detected in rat cerebral cortex, hippocampus, vas deferens, kidney, heart and prostate tissues known to contain the α_{1A}-adrenoceptor subtype, but not in tissues known to contain α_{1B}-adrenoceptor (spleen, liver) and α_{1D}-adrenoceptor (aorta). Scatchard analysis of [^{125}I]L-762,459 binding in rat cerebral cortex and prostate indicated a single binding site with a K_d of 0.7 nM and B_{max} of 11 (cerebral cortex) and 1 (prostate) pmole/g tissue. Specific and saturable [^{125}I]L-762,459 binding was also found in human cerebral cortex, liver, prostate and vas deferens (K_d = 0.2–0.4 nM, B_{max} = 0.4–4 pmole/g tissue). The specific binding in rat and human tissues was competed by non-selective α_1-adrenoceptor compounds (K_i values in nM: prazosin (0.14–1.2), terazosin (1.8–5.9) and phentolamine (2.4–11)) and selective α_{1A}-adrenoceptor compounds [K_i values in nM: (+) niguldipine (0.04–1.2) and SNAP 5399 ((±)-2-((2-aminoethyl)oxy)methyl-5-carboxamido-6-ethyl-4-(4-nitrophenyl)-3-*N*-(3-(4,4-diphenylpiperidin-1-yl)propyl)carboxamido-1,4-dihydropyridine hydrate (0.5–4.8)]. The results were consistent with the selective binding of [^{125}I]L-762,459 to the α_{1A}-adrenoceptor. The specific labeling of the α_{1A}-adrenoceptor subtype by [^{125}I]L-762,459 may make it a useful tool to localize the distribution of the α_{1A}-adrenoceptor.

Keywords: α_{1A}-Adrenoceptor; Binding; Radioligand; Adrenoceptor, subtype

1. Introduction

The heterogeneity of α_1-adrenoceptors was initially demonstrated by radioligand binding studies. Morrow and Creese (1986) first demonstrated competition of [^{3}H]prazosin binding in rat brain tissues by WB 4101 and phentolamine was best fit to a two-site model. The site having higher affinity for phentolamine, or WB4104 was termed α_{1A}-adrenoceptor, and the site having lower affinity for phentolamine or WB 4101 was designated α_{1B}-adrenoceptor. The existence of at least two α_1-adrenoceptor subtypes was further supported by the report that 5-methylurapidil (Gross et al., 1988) and (+) niguldipine (Boer et al., 1988) also had higher affinity for the α_{1A}-adrenoceptor subtype. The α_1-adrenoceptor heterogeneity was further revealed by showing selective inactivation of the α_{1B}-adrenoceptor subtype by alkylation with chloroethylclonidine (Han et al., 1987). Subsequent molecular cloning studies have identified three distinct subtypes: α_{1A}-adrenoceptor (also known as α_{1C}-adrenoceptor initially) (Schwinn et al., 1990; Forray et al., 1994; Hirasawa et al., 1993), α_{1B}-adrenoceptor (Cotecchia et al., 1988) and α_{1D}-adrenoceptor (Lomasney et al., 1991; Perez et al., 1991). (Nomenclature according to International Union of Pharmacology, Hieble et al., 1995.) The cloned α_{1A}-adren-

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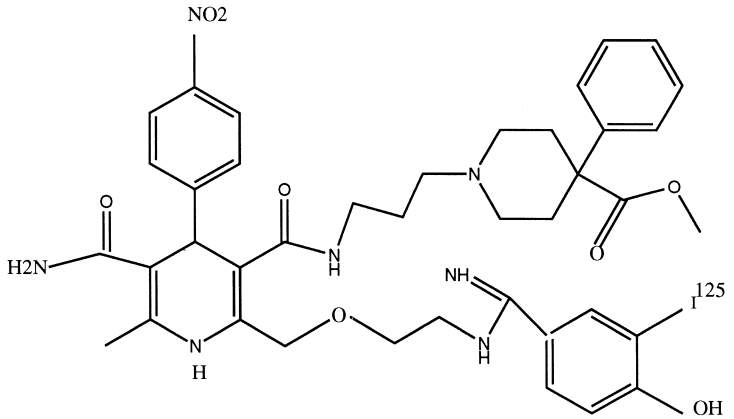

Fig. 1. Structure of [^{125}I]L-762,459.

oceptor is believed to be equivalent to the classical α_{1A}-adrenoceptor subtype (Laz et al., 1994; Perez et al., 1994), while the cloned α_{1B}-adrenoceptor corresponds to classical α_{1B}-adrenoceptor (Cotecchia et al., 1988) and the cloned α_{1D}-adrenoceptor corresponds to α_{1D}-adrenoceptor found predominantly in rat aorta (Goetz et al., 1995; Kenny et al., 1995).

The distribution of these α_1-adrenoceptor subtypes has been determined by radioligand binding studies using subtype selective antagonists or by determination of the levels of their mRNA in various tissues (Price et al., 1994). However, to facilitate the characterization of α_1-adrenoceptor subtypes, selective radioligands for each α_1-adrenoceptor subtypes are desirable.

All the radioligands used including [^{3}H]prazosin (Morrow and Creese, 1986), (±)-β-([^{125}I]iodo-4-hydroxyphenyl)-ethyl-aminomethyl-tetralone ([^{125}I]HEAT) (Han et al., 1987), [^{3}H]YM617 (tamsulosin) (Yazawa et al., 1992) and [^{3}H]WB 4101 (Morrow and Creese, 1986) were not α_1-adrenoceptor subtype selective or their selectivity for the α_{1A}-adrenoceptor subtype was not great. [^{3}H]5-Methylurapidil has been reported to selectively label the α_{1A}-adrenoceptor subtype, however, because of its known affinity for the 5-hydroxytryptamine (5-HT)$_{1A}$ receptor, the inclusion of 10 μM of 5-HT to mask its binding to the 5-HT receptor was required (Graziadei et al., 1989). In the present study, L-762,459 ((±)1-(3-{[5-carbamoyl-2-{2-[(4-hydroxy-3-iodobenzimidoyl)amino]ethoxy-methyl}-6-methyl-4-(4-nitro-phenyl)-1,4-dihydropyridine-3-carbonyl]amino}-propyl)-4-phenyl-1-piperidine-4-carboxylic acid methyl ester) (Fig. 1), an iodinated analog of a series of dihydropyridines previously reported to be selective α_{1A}-adrenoceptor antagonists, (Wetzel et al., 1995; Marzabadi et al., 1996) was found to have α_{1A}-adrenoceptor selectiv-

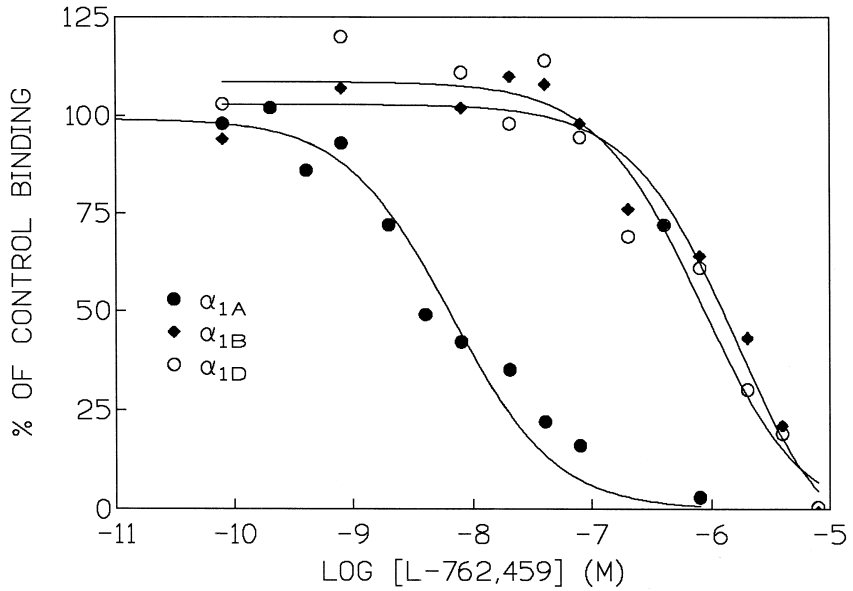

Fig. 2. Competition curves of [^{125}I]L-762,459 binding to three cloned human α_1-adrenoceptors by L-762,459. The results shown were obtained from means of four separate experiments performed in duplicates.

Table 1
The relative amount of specific [^{125}I]L-762,459 binding in rat tissues using a single concentration (0.1 nM) of [^{125}I]L-762,459

	fmol/g tissue
Cerebral cortex	1000 ± 170
Hippocampus	820 ± 120
Vas deferens	910 ± 190
Kidney	930 ± 270
Heart	350 ± 16
Prostate	110 ± 20
Urinary bladder	64 ± 16
Urethra	55 ± 6.6
Spleen	44 ± 17
Liver	33 ± 11
Aorta	13 ± 13

Values are mean ± S.E.M. from three separate determinations using tissue concentrations within the linear ranges.

ity. Here we report the characterization of specific binding of [^{125}I]L-762,459 as an α_{1A}-adrenoceptor selective radioligand in rat and human tissues.

2. Materials and methods

2.1. Materials

[^{125}I]L-762,459 (Fig. 1) (specific activity 1000–1800 Ci/mmole) was prepared in Merck Research Laboratories (B. Frances et al., manuscript in preparation). SNAP 5399 ((±)-2-((2-aminoethyl)oxy)methyl-5-carbox-amido-6-ethyl-4-(4-nitrophenyl)-3-*N*-(3-(4,4-diphenylpiperidin-1-yl)-propyl)carboxamido-1,4-dihydro-pyridine) was synthesized at Synaptic Pharmaceutical (Marzabadi et al., 1996). Other radioligands and chemicals were from commercial suppliers.

2.2. Preparation of tissue membranes

Fresh rat tissues were dissected free from fat and other adjacent tissues. Frozen human tissues were obtained from National Disease Research Interchange (NDRI) (Philadelphia, PA). All tissues were minced with scissors and homogenized with a polytron in TNE buffer (50 mM Tris–HCl, 5 mM disodium–EDTA (ethylenediaminetetraacetate), 150 mM sodium chloride pH 7.4). The homogenates were filtered using cheesecloth and the filtrates were centrifuged at 50 000 × g for 10 min. Membrane pellets were resuspended and washed by centrifugation as described above. The resulting pellets were resuspended in appropriate volumes of TNE buffer so that the specific [^{125}I]L-762,459 binding was linearly proportional to the tissue concentrations. Rat tissue membrane concentrations were 50–400 ml per gram of original tissue wet weight in the studies of rat tissue distribution of [^{125}I]L-762,459 binding. In other studies, the tissue membrane concentrations indicated in parenthesis as the volumes in ml added per gram of tissue wet weight were rat cerebral cortex (100), rat prostate (60), human cerebral cortex (100), human liver (100), human vas deferens (80) and human prostate (60).

2.3. [^{125}I]L-762,459 and [^{3}H]prazosin binding assays to tissue membranes

For binding assays 5–10 μl of dimethyl sulfoxide (for total binding), phentolamine (10 μM final concentration for non-specific binding) or test compounds (at various

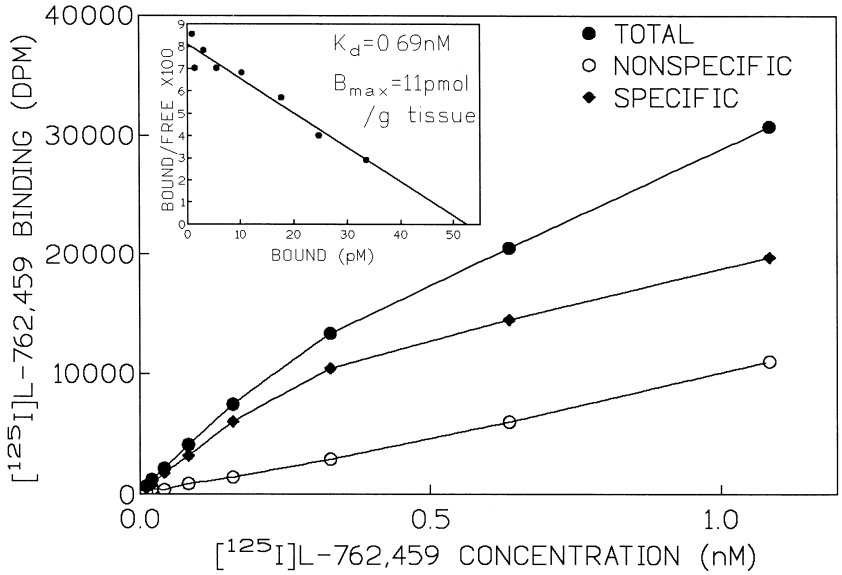

Fig. 3. Saturation of [^{125}I]L-762,459 binding to rat cerebral cortex. Inset, Scatchard plot. Each point represents the means of triplicate determinations from a single experiment. The experiments were replicated three times with similar results.

concentrations) and 10 μl of [^{125}I]L-762,459 (0.1–0.25 nM final concentration or unless indicated otherwise), or [^{3}H]prazosin (0.4 nM final concentration) and 250–500 μl of membranes were used. The reaction mixtures were incubated for 2 h at 37°C for [^{125}I]L-762,459 or 1 h at 25°C for [^{3}H]prazosin. The reaction was terminated by rapid filtration through glass–fiber filters (pre-soaked with 0.3% polyethleneimine) and washed immediately with TNE buffer. The binding assay results were analyzed with Graph-Pad Prism using one-site or two-site competition. K_i values were calculated according to the formula $K_i = IC_{50}/(1 + [L]/K_d)$, (Cheng and Prusoff, 1973).

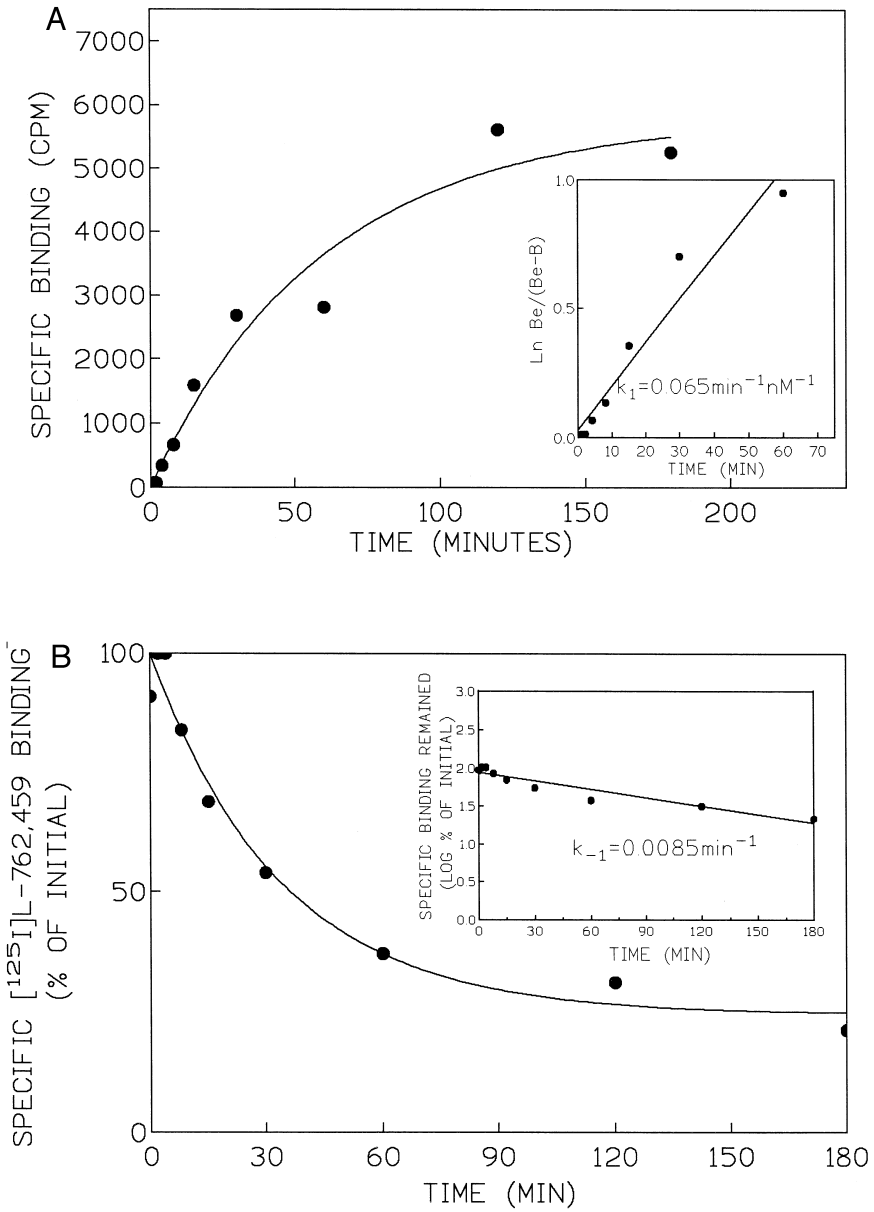

Fig. 4. Association and dissociation rates of [^{125}I]L-762,459 binding in rat cerebral cortex at 37°C. (A) Time course of association of [^{125}I]L-762,459 binding. Each point represents the mean of triplicate determinations from a single experiment. The experiments were replicated three times with similar results. Inset, pseudo-first-order kinetic plot of initial specific [^{125}I]L-762,459 binding. On the ordinate, B is the amount of specific binding at time t and Be is the amount of specific binding at equilibrium. The slope of the plot is the observed rate constant (k_{ob}) for the pseudo-first-order reaction. The second-order association rate is calculated from $k_1 = (k_{ob} - k_{-1})/\{[^{125}\text{I}]\text{L-762,459}\}$. $\{[^{125}\text{I}]\text{L-762,459}\}$ is the concentration of radioligand used in the experiment and k_{-1} is obtained from panel B. (B) Dissociation of specific [^{125}I]L-762,459 binding. For dissociation studies, [^{125}I]L-762,459 was allowed to associate as described in panel A for 2 h, whereupon 10 μM (final concentration) phentolamine was added to prevent rebinding of dissociated [^{125}I]L-762,459. Dissociation was determined at various times after the addition of phentolamine. Each point represents the mean of triplicate determination from a single experiment. The experiments were replicated three times with similar results. The data were plotted as a linear plot and a semilogarithmic plot (inset) of specific [^{125}I]L-762,459 binding expressed as percentage of initial binding at equilibrium (y-axis, log values) and the time after the addition of phentolamine (x-axis). The dissociation rate constant (k_{-1}) was calculated according to the formula $k_{-1} = 2.3 \times$ slope (inset).

2.4. [^{125}I]HEAT binding to cloned α_1-adrenoceptor membranes

DNAs encoding the human α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors were cloned by homology to the published sequences from other species (Weinberg et al., 1994). α_{1A}-, α_{1B}-, and α_{1D}-adrenoceptors were stablely expressed in Chinese Hamster Ovary (CHO), Mouse Fibroblast (LM) and Human Embryonic Kidney (HEK293) cells, respectively. Membranes were prepared from these cells by washing the cells in phosphate buffered saline, detaching the cells from the plate using enzyme-free dissociation solution (Specialty Media, Lavallette, NJ), then resuspending the cells in 50 mM Tris–HCl pH 7.4, 5 mM EDTA plus protease inhibitors (4 μg/ml leupeptin, 10 μM phosphoramidine, 40 μg/ml bacitracin, 5 μg/ml aprotinin and 1 mM AEBSF (4-(2-aminoethyl)benzenesulfonyl fluoride). The resuspended cells were disrupted by polytron and the membranes pelleted at 50,000 × g for 15 min. The membranes were then resuspended in 50 mM Tris–HCl pH 7.4, 5 mM EDTA, 150 mM NaCl (TNE) plus protease inhibitors, passed through a 26 gauge needle to homogenize, quick frozen in liquid nitrogen and stored at −70°C. [^{125}I]HEAT binding reaction was performed in duplicate using TNE buffer in a final volume of 200 μl consisting 0.1 nM of [^{125}I]HEAT with or without various concentra-

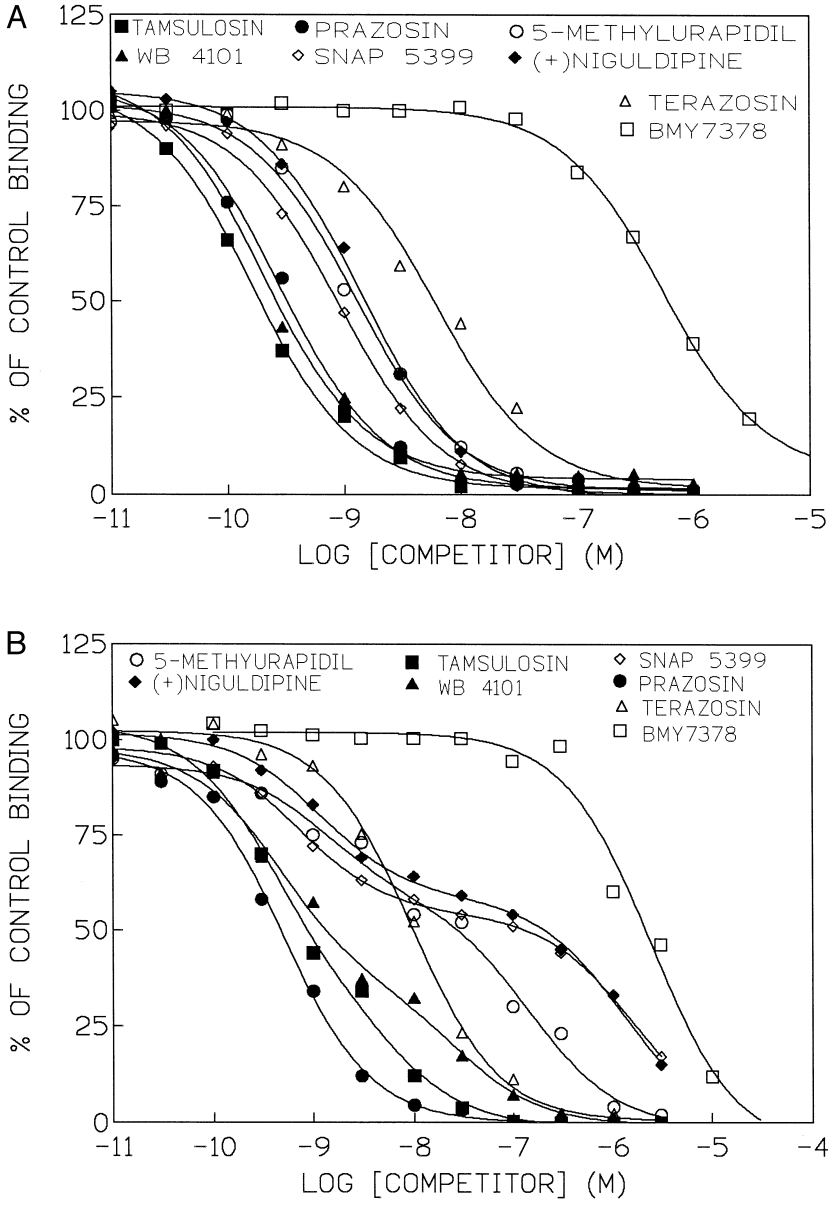

Fig. 5. Competition studies of specific [^{125}I]L-762,459 binding and [^{3}H]prazosin binding by various α_1-adrenoceptor antagonists in rat cerebral cortex. Each point represents mean from three determinations. In panel A ([^{125}I]L-762,459 binding), all curves were best fit to one-site competition model, while in panel B ([^{3}H]prazosin binding), tamsulosin, WB 4101, (+) niguldipine, 5-methylurapidil and SNAP 5399 were fit to two-site competition model significantly ($P < 0.05$) better.

tions of competing compounds. Binding reaction was at room temperature for 1 h with shaking. The reaction mixtures were filtered onto Wallac Printed Filtermat B glass–fiber filters using a Tomtec harvester and washed with 50 mM Tris, pH 7.4. The radioactivity on the filters was determined in a Wallac β-plate counter. IC_{50} values and K_i values were obtained as described above in Section 2.3.

3. Results

3.1. Selectivity of nonradioactive L-762,459 on cloned human α_1-adrenoceptor subtypes

The competition of specific [^{125}I]HEAT binding to the three cloned human α1-adrenoceptors by unlabeled L-762,459 were shown in Fig. 2. The K_i values were calculated to be 1.3 ± 0.2, 240 ± 38 and 280 ± 48 nM ($n = 4$) for α_{1A}-, α_{1B}-, and α_{1D}-adrenoceptors.

3.2. Distribution of specific [^{125}I]L-762,459 binding in rat tissues

Specific [^{125}I]L-762,459 binding was detected in various rat tissues with high amounts found in cerebral cortex, hippocampus, vas deferens and kidney followed by heart and prostate (Table 1). Low specific [^{125}I]L-762,459 binding was observed in rat spleen, liver and aorta.

3.3. Saturation analysis of [^{125}I]L-762,459 binding in rat cerebral cortex and prostate

Specific [^{125}I]L-762,459 binding to rat cerebral cortex (Fig. 3) and rat prostate (data not shown) was saturable. Scatchard analysis (inset of Fig. 3) indicated a single class of binding site with a K_d value of 0.69 ± 0.04 nM and B_{max} of 11 ± 3 pmol/g tissue in rat cerebral cortex (Fig. 3) and with a K_d value of 0.69 ± 0.07 nM and B_{max} of 1.1 ± 0.05 pmol/g tissue in rat prostate (data not shown). It should be noted that the highest concentration of [^{125}I]L-762,459 used in the saturation studies was only $2\times$ the K_d. However, competition studies of specific [^{125}I]L-762,459 binding in rat cerebral cortex using a wider concentration range of unlabeled L-762,459 (0.01–100 nM) and analysis of the data using Ligand-EBDA (cold saturation) also yielded a single class of binding sites with comparable K_d (0.76 ± 0.18 nM) and B_{max} (6.5 ± 1.5 pmol/g tissue) (data not shown).

3.4. Kinetics of [^{125}I]L-762,459 binding in rat cerebral cortex

Specific [^{125}I]L-762,459 binding to rat cerebral cortex was time-dependent and reached a steady-state at approximately 2 h (Fig. 4A). The calculated association rate constant (k_1) was 0.065 min^{-1} nM^{-1} (Fig. 4A inset).

The rate of dissociation of [^{125}I]L-762,459 binding was examined by incubating [^{125}I]L-762,459 to equilibrium and then adding 10 μM of phentolamine to prevent rebinding of dissociated [^{125}I]L-762,459. The remaining bound [^{125}I]L-762,459 was measured at various time intervals (Fig. 4B). When plotted in a semi-logarithmic scale, the dissociation was linear indicating a first-order process (Fig. 4B inset). The dissociation rate constant (k_{-1}) was calculated to be 0.0085 min^{-1}. The dissociation constant (K_d)

Table 2
K_i (competitor dissociation constant) values in nM obtained from competitive binding studies of specific [^{125}I]L-762,459 and [^{3}H]prazosin binding to rat cerebral cortex and prostate membranes

	Cerebral cortex		Prostate	
	[^{125}I]L-762,459	[^{3}H]Prazosin	[^{125}I]L-762,459	[^{3}H]Prazosin
Prazosin	0.27 ± 0.037	0.18 ± 0.014	0.24 ± 0.036	0.15 ± 0.069
5-Methylurapidil	1.1 ± 0.13	1.3 (39%) 66 (61%)	0.95 ± 0.030	0.66 ± 0.18 (76%) 38 ± 7.2 (24%)
(+) Niguldipine	1.2 ± 0.032	0.44 ± 0.28 (43%) 690 ± 410 (57%)	0.72 ± 0.14	0.25 ± 0.045 (72%) 144 ± 70 (28%)
WB 4101	0.16 ± 0.026	0.18 (56%) 9.0 (44%)	0.25	0.13 ± 0.022
Terazosin	5.8 ± 0.92	1.8	1.8	3.7 ± 1.3
Tamsulosin	0.094 ± 0.040	0.086 ± 0.035 (55%) 1.4 ± 0.42 (45%)	0.035	0.026 ± 0.0029 (79%) 1.0 ± 0.093 (21%)
SNAP 5399	0.63 ± 0.15	0.19 ± 0.050 (44%) 590 ± 210 (56%)	0.54 ± 0.084	0.17 ± 0.050 (76%) 240 ± 99 (24%)
BMY 7378	650 ± 180	230 ± 100	170	150 ± 65
Norepinephrine	$12{,}000 \pm 1400$	6000		

Values in parentheses are percentages of high and low affinity sites when the curves are best-fit to two-site model.
Values are means $\pm$ S.E.M. from three separate determinations.
Values without S.E.M. are means from two determinations.
K_i values were calculated according to the following formula $K_i = IC_{50}/(1 + L/K_d)$, where L is the radioligand concentration and K_d the dissociation constant of radioligand.
IC_{50} values were obtained from the competition data and analyzed with the Graph-Pad Prism program using one-site or two site competition.

Table 3
K_i (competitor dissociation constant) values in nM from competitive binding studies of specific [^{125}I]L-762,459 binding to human tissue membranes and [^{125}I]HEAT binding to cloned human α_1-adrenoceptor subtypes and K_d and B_{max} of specific [^{125}I]L-762,459 binding in human tissues

	[^{125}I]L-762,459 binding				[^{125}I]HEAT binding		
	Cerebral cortex	Liver	Prostate	Vas deferens	α_{1A}	α_{1B}	α_{1D}
Prazosin	0.19	0.24	0.14	0.42	0.39 ± 0.21	0.21 ± 0.045	0.20 ± 0.059
(+) Niguldipine	0.18	0.23	0.081	0.039	0.54 ± 0.028	67 ± 8.5	110 ± 28
WB 4101	0.38	0.70	0.33	1.4	0.39[a]	4.5[a]	0.91[a]
SNAP 5399	1.6	4.8	1.9	2.2	0.61 ± 0.17	240 ± 24	640 ± 130
Tamsulosin			0.022	0.060	0.090 ± 0.011	0.60 ± 0.28	0.44 ± 0.27
5-methylurapidil	2.6	12	1.8		2.1[a]	170[a]	12[a]
Terazosin	5.3	3.7	5.9		4.2 ± 0.46	1.2 ± 0.15	2.0 ± 0.63
Phentolamine	5.5	7.8	11	6.5	7.7 ± 3.1	51 ± 7.2	110 ± 65
BMY 7378	220		440		410 ± 110	160 ± 14	2.8 ± 0.13
Norepinephrine	11,000		8500				
K_d (nM)	0.35	0.20	0.30 ± 0.05	0.26			
B_{max} (pmd/g tissue)	3.8	2.9	0.90 ± 0.15	0.38			

K_i values were calculated as described in Table 2 legend.
K_i values in human tissues without S.E.M. were mean of two separate determinations which differed from each other by 10–190%.
K_i values in cloned human α_1-adrenoceptor subtypes were mean ± S.E.M. from three or more determinations.
B_{max} and K_d were obtained from Scatchard plots from three experiments. (Values without S.E.M. were from two separate experiments which differ from each other by 10–90%).
[a]Data from Forray et al. (1994) cited in the reference list.

determined from the ratio k_{-1}:k_1 was 0.13 nM, slightly lower than the K_d determined in equilibrium studies.

3.5. Effect of α_1-adrenoceptor agonist and antagonists on specific [^{125}I]L-762,459 binding to rat cerebral cortex and prostate

Specific [^{125}I]L-762,459 binding to rat cerebral cortex and prostate was competed by α_1-adrenoceptor subtype non-selective and selective compounds in a monophasic manner indicating a single class of binding sites labeled by [^{125}I]L-762,459 (Fig. 5A and Table 2). All of the α_1-adrenoceptor agents examined competed with the [^{125}I]L-762,459 binding to the same maximal level defined by 10 μM of phentolamine (nonspecific binding) (Fig. 5A). The results were in contrast to the biphasic competition of [^{3}H]prazosin binding by some α_{1A}-adrenoceptor subtype selective antagonists (5-methylurapidil, (+) niguldipine, tamsulosin, WB 4101, and SNAP 5399) (Fig. 5B and Table 2). Specific [^{125}I]L-762,459 binding was weakly competed by BMY 7378 (K_i = 170–680 nM), an α_{1D}-adrenoceptor selective antagonist, in rat cerebral cortex and prostate (Table 2). The K_i values for specific [^{125}I]L-762,459 binding in these tissues were in agreement with the K_i values of

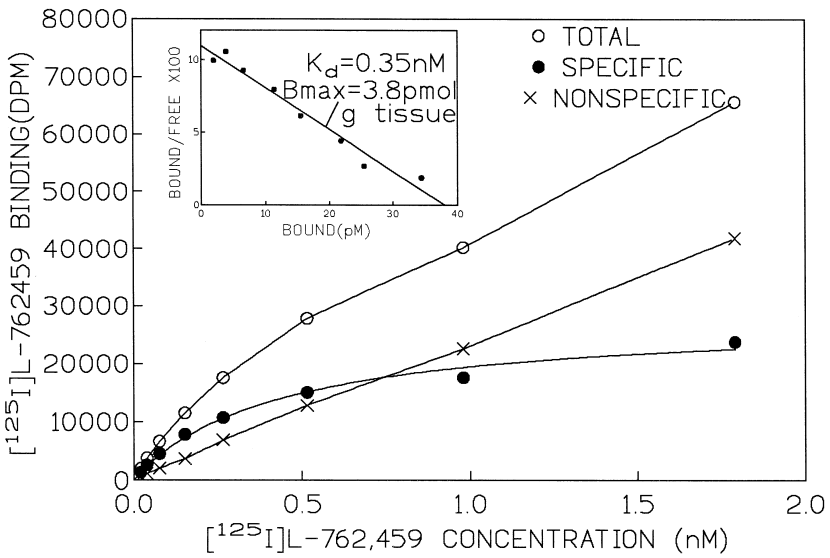

Fig. 6. Saturation of [^{125}I]L-762,459 binding to human cerebral cortex. Inset, Scatchard plot. Each point represents means of triplicate determinations from a single experiment. The experiment was repeated with similar results.

[^{3}H]prazosin binding for α_1-adrenoceptor subtype nonselective compounds and with the K_i values of the high affinity site (i.e. α_{1A}-adrenoceptor) for the α_{1A}-adrenoceptor selective antagonists [correlation coefficient (r) of $-\log K_i$: 0.99 and 0.98 for cerebral cortex and prostate, respectively] (Table 2).

3.6. Specific [^{125}I]L-762,459 binding in human tissues

Specific and saturable [^{125}I]L-762,459 binding was also observed in human tissues with K_d values of 0.20–0.35 nM and B_{max} of 0.38–3.8 pmol/g tissue (Table 3). Representative saturation study was shown in Fig. 6.

Specific [^{125}I]L-762,459 binding to human tissues was competed by α_{1A}-adrenoceptor selective and non-selective compounds monophasically. The potencies corresponded to their affinities on the α_{1A}-adrenoceptor (Table 3), since the K_i values were similar to the values for specific [^{125}I]HEAT binding to the cloned human α_{1A}-adrenoceptor (r vs. cerebral cortex = 0.96, prostate = 0.96, $p < 0.001$). The K_i values were distinct from the values for α_{1B}- (r vs. cerebral cortex = 0.52, prostate = 0.62; $p > 0.05$) and/or α_{1D}-adrenoceptor (r vs. cerebral cortex = 0.03, prostate = 0.22; $p > 0.05$) for α_1-adrenoceptor subtype selective compounds (Table 3).

4. Discussion

L-762,459 is a racemate consisting of 1:1 ratio of two enantiomers. No attempt was made to resolve the two enantiomers, though previous study of the same series of compounds indicated the stereoselectivity with the (−) enantiomer substantially more active toward α_{1A}-adrenoceptor (Wetzel et al., 1995).

The binding of [^{125}I]L-762,459 to rat cerebral cortical membranes is reversible and saturable. Scatchard plot analysis indicated a single class of binding sites for [^{125}I]L-762,459 with high affinity (K_d = 0.20–0.69 nM) in rat and human tissues examined. The B_{max} of specific [^{125}I]L-762,459 binding in rat cerebral cortex was approximately 60% of [^{3}H]prazosin binding when parallel experiments were done on the same preparation (O'Malley, unpublished data). The B_{max} of specific [^{125}I]L-762,459 binding (7–11 pmol/g tissue) was slightly lower than those (14 pmol/g tissue) of [^{3}H]prazosin binding reported by others in this tissue (Morrow and Creese, 1986). Lower B_{max} of [^{125}I]L-762,459 binding than the B_{max} of [^{3}H]prazosin binding was expected since [^{3}H]prazosin labeled all α-adrenoceptors while [^{125}I]L-762,459 only labeled α_{1A}-adrenoceptor. Specific [^{125}I]L-762,459 binding to all tissues examined was competed by α_{1A}-adrenoceptor subtype nonselective and selective compounds in a monophasic manner indicating a single class of binding sites labeled by [^{125}I]L-762,459. The results were in contrast to the biphasic competition of [^{3}H]prazosin binding by the α_{1A}-adrenoceptor subtype selective antagonists in some tissues (rat prostate and cerebral cortex) known to contain α_{1A}- and non-α_{1A}-adrenoceptor subtypes. The K_i values of α_{1A}-adrenoceptor subtype nonselective compounds for [^{125}I]L-762,459 binding were in agreement with the values of specific [^{3}H]prazosin binding. Moreover, the K_i values for specific [^{125}I]L-762,459 binding using the α_{1A}-adrenoceptor subtype selective compounds were also similar to the values for the high affinity site of [^{3}H]prazosin binding in tissues (i.e. α_{1A}-adrenoceptor subtypes) (Table 2) and the values of [^{125}I]HEAT binding to cloned human α_{1A}-adrenoceptor (Table 3).

The relative distribution of specific [^{125}I]L-762,459 binding is high in rat tissues (cerebral cortex, vas deferens, kidney, heart and prostate) which have been reported to contain α_{1A}-adrenoceptor subtype based on the high affinity ($pK_i > 9$) of WB 4101 and resistance to chlorethylclonidine inactivation (Minneman et al., 1988; Yazawa and Honda, 1993). In contrast, specific [^{125}I]L-762,459 binding to rat liver and spleen (tissues known to contain predominantly α_{1B}-adrenoceptor (Han et al., 1987; Burt et al., 1995)) and rat aorta (a tissue known to contain mainly α_{1D}-adrenoceptor (Goetz et al., 1995; Kenny et al., 1995)) was low. Similarly, specific [^{125}I]L-762,459 binding was detected in human tissues reported to contain α_{1A}-adrenoceptor subtype including human cerebral cortex (Gross et al., 1988), liver (Garcia-Sáinz et al., 1995), prostate (Forray et al., 1994) and vas deferens (Furukawa et al., 1995) using radioligand binding and/or functional studies.

5. Conclusions

In conclusion, the results taken together were consistent with the selective binding of [^{125}I]L-762,459 to α_{1A}-adrenoceptor. Specific labeling of the α_{1A}-adrenoceptor subtype by [^{125}I]L-762,459 makes it a useful tool to localize the distribution of the α_{1A}-receptor. The autoradiographic studies in rat brain (Gibson et al., manuscript in preparation) and urinary bladder of various species (Durkin et al., 1997) supported this contention.

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