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Characterization of the Binding of [^{3}H]L-158,809: A New Potent and Selective Nonpeptide Angiotensin II Receptor (AT_1) Antagonist Radioligand

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SUMMARY

[^{3}H]L-158,809, a new potent and AT_1-selective nonpeptide angiotensin II receptor antagonist, bound saturably and reversibly to rat adrenal membranes. Scatchard and Hill plot analyses indicated a single class of high affinity (K_d = 0.66 nM) binding sites. The relative potencies of various angiotensin II-related peptide and nonpeptide antagonists in displacing [^{3}H]L-158,809 binding correlated with their potencies in displacing the binding of ^{125}I-Sar^1,Ile^8-angiotensin II to adrenal AT_1 receptors. [^{3}H]L-158,809 binding to adrenal membranes was not affected by addition of guanosine-5′-(β,γ-imido)triphosphate or various pharmacological agents known to interact with other common peptide and nonpeptide receptor systems. The potencies of angiotensin II receptor agonists, but not antagonists, in inhibiting specific [^{3}H]L-158,809 binding were decreased in the presence of guanosine-5′-(β,γ-imido)triphosphate. Specific [^{3}H]L-158,809 binding was also observed in rat liver and kidney. Collectively, the data indicate that [^{3}H]L-158,809 represents a new, potent, nonpeptide, antagonist radioligand suitable for the study of angiotensin II AT_1 receptors.

Recently, two subtypes of AII receptors have been identified based upon their differential affinities for the nonpeptidyl antagonists DuP-753 (MK-954; losartan, DuP-89; Example 89, Example No. 90), WL-19 (PD121981), and Exp 655 (PD123177) and the peptide CGP 42112A (1–4). The AII receptor subtype having a high affinity for DuP-753 has been designated as AT_1. The other AII receptor subtype has a high affinity for WL-19, Exp 655, and CGP 42112A and has been designated as AT_2 (5). The relative distribution and abundance of AII receptor subtypes are tissue and species dependent (2, 6).

To facilitate the characterization of AII receptor subtypes, selective radioligands are desirable. DuP-753, a selective AT_1 receptor antagonist (7), has been radiolabeled and used as a radioligand (8). Recently, a structurally novel, nonpeptide, AT_1-selective, competitive antagonist, L-158,809, was identified that has a much higher affinity than DuP-753 for AT_1 receptors *in vitro* (9–11). In the present studies, we have characterized the properties of [^{3}H]L-158,809 as an AT_1-selective radioligand in the rat adrenal, liver, and kidney.

Materials and Methods

Radioligands. ^{125}I-Sar^1,Ile^8-AII was purchased from New England Nuclear. [^{3}H]L-158,809 (8.4 Ci/mmol) (Fig. 1) was prepared by chemists at Merck Research Laboratories.[1]

Binding assays. Membranes from rat whole adrenal (in some regional distribution studies adrenal was separated into capsular and decapsulated tissue), kidney cortex, and liver were prepared by homogenization in 50 mM Tris·HCl (pH 7.7) and centrifuged at 50,000 × *g* for 10 min. The resulting pellets were washed twice in 120 mM NaCl, 5 mM EDTA, 10 mM Na_2HPO_4, 0.1 mM phenylmethanesulfonyl fluoride (pH 7.4), by resuspension and centrifugation. The membrane pellets were resuspended in appropriate volumes (500, 100, and 200 volumes for adrenal, kidney, and liver, respectively) of binding assay buffer (120 mM NaCl, 10 mM Na_2HPO_4, 5 mM EDTA, 0.1 mM phenylmethanesulfonyl fluoride, 0.2 mg/ml soybean trypsin inhibitor, 0.018 mg/ml *o*-phenanthroline, 2 mg/ml heat-denatured bovine serum albumin, 0.14 mg/ml bacitracin, pH 7.4). To measure specific [^{3}H]L-158,809 binding, 1 ml of membranes was added to triplicate tubes containing 10 μl of either buffer (for total binding) or unlabeled L-158,809 or Sar^1,Ile^8-AII (1 μM final concentration for nonspecific binding) or displacers (at desired final concentrations) and 10 μl of [^{3}H]L-158,809 (0.5–1 nM final concentration, unless indicated otherwise). After incubation at 37° for 60 min (various intervals were used in association rate study), the incubation mixtures were filtered through glass fiber GF/B filters (presoaked in 0.1% bovine serum albumin in 5 mM Tris·HCl, pH 7.4, 0.15 M NaCl) and rapidly washed four times with 4 ml of ice-cold Tris·HCl (5 mM, pH 7.4) containing 0.15 M NaCl. The radioactivity trapped on the filters was counted by liquid scintillation counting. ^{125}I-Sar^1,Ile^8-AII binding to rat adrenal, kidney, and liver was performed as reported previously (6).

Functional assays. The methods used for evaluation of the effect of antagonists upon AII-induced contractions of the isolated rabbit

[1] R. A. Rivero, P. K. Chakravarty, R. Chen, W. J. Greenlee, A. Rosegay, and R. Simpson. The synthesis of [^{3}H] Losartin, [^{3}H] L-158,641, and [^{3}H] L-158,809. Manuscript in preparation.

ABBREVIATIONS: AII, angiotensin II; AIII, angiotensin III; Gpp(NH)p, guanosine-5′-(β,γ-imido)triphosphate; TLC, thin layer chromatography.

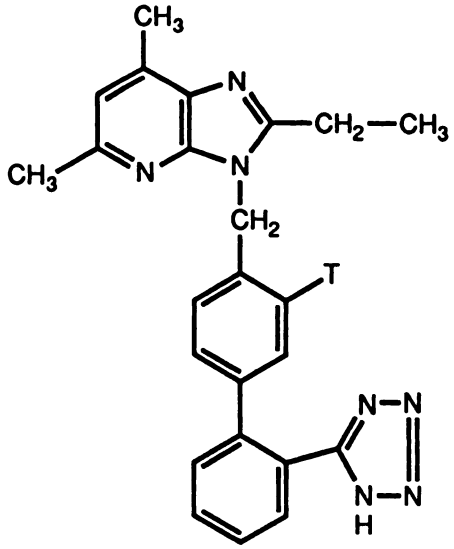

Fig. 1. Structure of [^{3}H]L-158,809. *T*, tritium.

aorta were as described previously (10). K_b values were determined from Schild plots (−log pA_2) or double-reciprocal plots as described by Kenakin (12). The former method was used for antagonists (L-158,338, DuP-753, and SKF108566), which produced parallel shifts in the AII concentration-response curves, did not significantly affect AII maximal contractile responses, and gave Schild plot slopes not significantly different from unity. The latter method was used for antagonists (L-158,809 and EXP3174), which significantly reduced AII maximal contractile responses.

Stability of [^{3}H]L-158,809. To determine the stability of [^{3}H]L-158,809 during incubation in the binding assays, [^{3}H]L-158,809 (2 nM) was incubated with adrenal, kidney, or liver membranes at 37° for 60 min as described for other binding assays, with the exception that only a 0.5-ml final volume was used. At the end of the incubation, 2.5 ml of methanol were added. After mixing vigorously, the mixtures were centrifuged at 1000 × *g* for 10 min. Supernatants were removed and dried with a Speed Vac concentrator. The dried residues were dissolved in 200 μl of methanol and 20-μl aliquots were applied to TLC plates (polyester silica gel, 20 × 20 cm; Sigma, St. Louis, MO). The TLC plates were developed in solvent 1 (chloroform/methanol/concentrated ammonia, 160:40:1) or solvent 2 (methylene chloride/acetic acid/methanol, 93:4:3). The developed TLC plates were cut into 0.5-cm pieces and their radioactivities were determined by liquid scintillation counting.

Results

Regional distribution of specific [^{3}H]L-158,809 binding in rat adrenal. Rat adrenals were dissected into adrenal capsular (mainly cortical glomerulosa cells) and decapsulated (medulla and remaining cortical cells) portions. Specific [^{3}H]L-158,809 binding (at 1 nM) was about 5 times higher in adrenal capsules, compared with the decapsulated portions (38 ± 2.8 versus 7.1 ± 1.1 fmol/20 mg of tissue), whereas nonspecific binding in the two regions was similar (2.9 ± 0.76 versus 2.9 ± 0.16 fmol/20 mg of tissue). The much higher concentration of [^{3}H]L-158,809 binding in adrenal capsules, compared with decapsuled portions, is consistent with previous reports that AT_1 receptors predominate in the rat adrenal capsules and AT_2 receptors predominate in the decapsulated portion (3). The whole rat adrenals were subsequently used in our studies because of convenience and the only slight improvement in the ratio of total to nonspecific binding obtained using the dissected capsules, compared with whole adrenals.

Tissue concentration linearity. The specific [^{3}H]L-158,809 binding increased linearly with the concentration of rat whole adrenal membranes up to at least 4 mg of wet tissue weight (data not shown). A tissue concentration of 2–4 mg/ml was subsequently used for routine binding studies.

Saturation analysis of [^{3}H]L-158,809 binding. The binding of [^{3}H]L-158,809 to whole rat adrenal membranes was saturable (Fig. 2A). The ratio of total binding to nonspecific binding was about 10 at a concentration of 1 nM, which was used for routine binding assays. Scatchard analysis (13) of specific [^{3}H]L-158,809 binding at various concentrations of [^{3}H]L-158,809 (0.05–6 nM) indicated a single class of binding sites with a dissociation constant of 0.66 ± 0.14 nM (Fig. 2B). The maximal number of binding sites for specific [^{3}H]L-158,809 binding was 20 ± 2.9 pmol/g of tissue, which is almost identical to the maximal number of AT_1 binding sites determined using ^{125}I-AII reported previously (3). A Hill (14) plot of the [^{3}H]L-158,809 binding data gave a Hill coefficient of 0.97 ± 0.04 (data not shown), indicating a single class of binding sites and the absence of positive or negative cooperative interaction.

Kinetics of [^{3}H]L-158,809 binding. The specific binding of [^{3}H]L-158,809 to rat adrenal membranes was rapid, time-dependent, reversible, and saturable. It reached steady state in approximately 30 min (Fig. 3A). The calculated association rate constant (k_1) was 0.070 ± 0.0086 min^{-1} nM^{-1} (Fig. 3B). The rate of dissociation was examined by incubating membranes with [^{3}H]L-158,809 to equilibrium and then adding 1 μM unla-

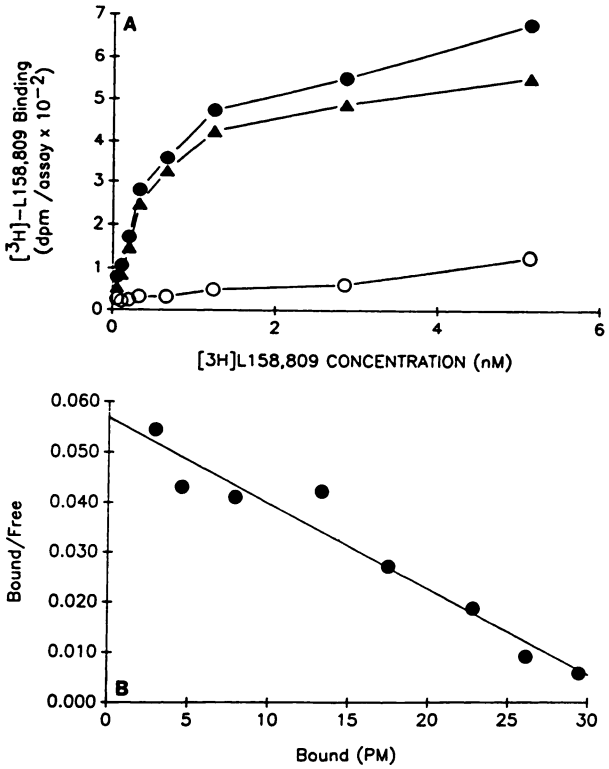

Fig. 2. [^{3}H]L-158,809 binding as a function of increasing concentrations of [^{3}H]L-158,809 in rat adrenal membranes. The binding assays were performed as described in Materials and Methods, using various concentrations of [^{3}H]L-158,809. The *points* shown are means of triplicate determinations. These experiments were replicated three times with similar results. A, ●, Total binding; ○, nonspecific binding; ▲, specific binding. B, Scatchard plot for specific [^{3}H]L-158,809 binding. The mean ± standard error of the K_d value and estimated maximal number of binding sites from three experiments are given in the text.

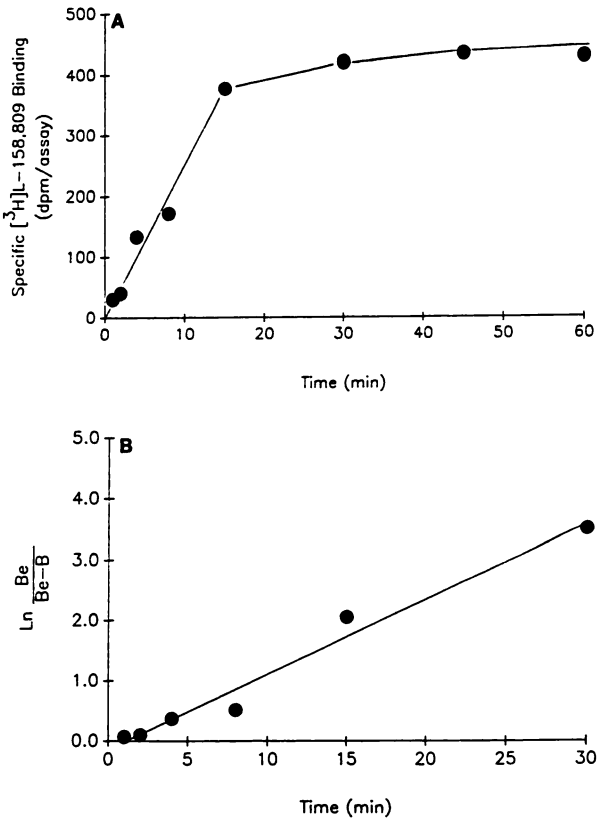

Fig. 3. Time course of association of [^{3}H]L-158,809 binding. The association of [^{3}H]L-158,809 binding to rat adrenal membranes was determined at various time intervals as described in Materials and Methods. Specific binding was defined as the difference between binding obtained in the absence and presence of 1 μM L-158,809. The *points* shown are those obtained in a single experiment, performed in triplicate. The experiments were replicated three times with similar results. A, Specific [^{3}H]L-158,809 binding as a function of time. B, Pseudo-first-order kinetic plots of initial specific [^{3}H]L-158,809 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, k_1, was calculated from $k_1 = (k_{ob} - k_{-1})/[[^3H]L\text{-}158,809]$. k_{-1} is the dissociation rate constant determined in Fig. 4 and [[^{3}H]L-158,809] is the concentration of radioligand used in the experiment.

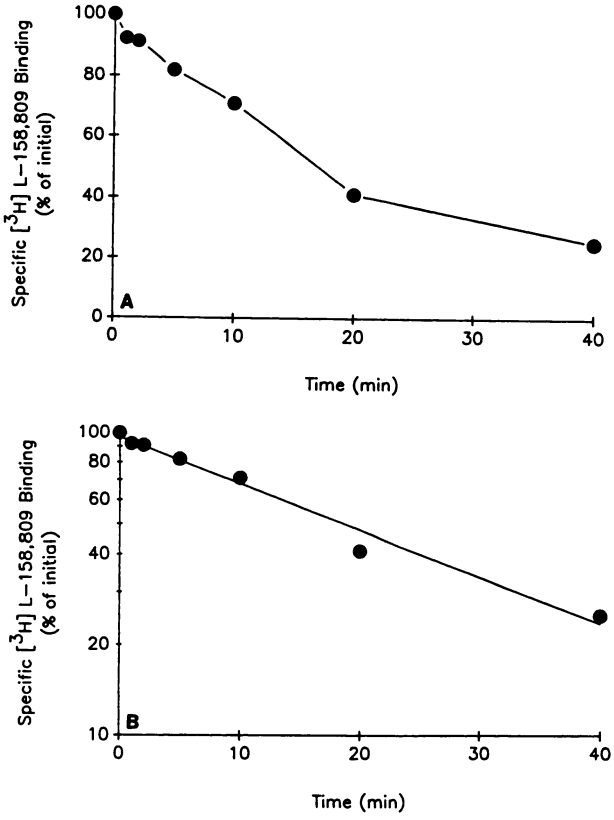

Fig. 4. Dissociation of specific [^{3}H]L-158,809 binding to rat adrenal membranes. The *points* shown were obtained in a single experiment, performed in triplicate. The experiments were replicated three times with similar results. For dissociation studies, [^{3}H]L-158,809 was allowed to associate for 60 min at 37°, whereupon 1 μM unlabeled L-158,809 was added to prevent rebinding of dissociated [^{3}H]L-158,809. The dissociation reaction was measured at various time intervals after the addition of unlabeled L-158,809, as described in Materials and Methods. Shown are a linear plot (A) and a semilogarithmic plot (B) of B/B_e, where B_e and *B* are binding at equilibrium and time *t* and *t* is the time after the addition of L-158,809. The dissociation rate constant (k_{-1}) was calculated according to the formula $k_{-1} = 2.3 \times$ slope.

beled L-158,809 to prevent rebinding of dissociated [^{3}H]L-158,809. The remaining bound [^{3}H]L-158,809 was measured at different time intervals (Fig. 4A). When plotted on a semilogarithmic scale, the dissociation was linear, indicating a first-order process (Fig. 4B). The dissociation rate constant (k_{-1}) was calculated to be 0.053 ± 0.011 min^{-1}. The dissociation constant determined from the ratio of k_{-1}/k_1 was 0.76 nM, similar to the dissociation constant determined in equilibrium studies.

Effect of AII agonists and antagonists on specific [^{3}H] L-158,809 binding in rat adrenal. The specific binding of [^{3}H]L-158,809 to rat adrenal membranes was inhibited by AII agonists and antagonists. The K_i values for AII antagonists, including Sar^1,Ile^8-AII, L-158,809 (9, 10), L-158,338 (9), EXP3174 (15), SKF 108566 (16), and DuP-753 (MK-954) (7), for inhibiting [^{3}H]L-158,809 binding were similar to their K_i values for inhibiting ^{125}I-Sar^1,Ile^8-AII binding to AT_1 receptors in rat adrenal (Table 1). The K_i value for L-158,809 (0.4 nM) was also in good agreement with the K_d (0.66 nM) determined using Scatchard analysis of [^{3}H]L-158,809 binding. The AT_2-selective ligand PD121981 (3) had no effect on specific [^{3}H]L-158,809 binding (Table 1). The K_i value of AII determined using inhibition of specific [^{3}H]L-158,809 binding was similar to its K_i obtained using ^{125}I-Sar^1,Ile^8-AII. AIII appeared to be somewhat less potent in inhibiting specific [^{3}H]L-158,809 binding than ^{125}I-Sar^1,Ile^8-AII binding (Table 1).

The rank order of potencies of the nonpeptide AII antagonists for inhibition of [^{3}H]L-158,809 binding (Tables 1 and 2) was in good agreement with their rank order of potencies in antagonizing AII-induced contractions of the isolated rabbit aorta (L-158,809 > L-158,338 = EXP3174 > SKF108566 > DuP-753 ≫ PD-121981) (Table 1).

Effect of various pharmacological agents on [^{3}H]L-158,809 binding in rat adrenal. Other pharmacological agents, including α-adrenergic, β-adrenergic, cholinergic, serotonergic, and dopaminergic agonists and/or antagonists, and the endogenous peptides bradykinin, substance P, vasopressin, and vasoactive intestinal peptide had no effect on specific [^{3}H]L-158,809 binding at concentrations generally considered to be pharmacologically effective (1 μM) (Table 3).

[^{3}H]L-158,809 binding to other tissues. The specific binding of [^{3}H]L-158,809 was also observed in rat kidney and

TABLE 1

Displacement of specific [^{3}H]L-158,809 and ^{125}I-Sar^1, Ile^8-AII (AT$_1$) binding in rat adrenal membranes and antagonism of AII contractions in the rabbit aorta by various AII agonists and/or antagonists

Values are mean ± standard error of at least three experiments. Values without standard error were obtained from one or two experiments. K_b values in parentheses are 95% confidence limits. K_i values were calculated according to the formula $K_i = IC_{50}/(1+ [L]/K_d)$, where $[L]$ is the radioligand concentration and K_d is the dissociation constant of the radioligand.

	K_i		K_b rabbit aorta[b]
	[^{3}H]L-158,809	^{125}I-Sar^1, Ile^8-AII (AT$_1$)[a]	
	nM		nM
AII	1.7 ± 0.37	1.6 ± 0.37	
AIII	10 ± 1.7	2.9 ± 0.73	
Sar^1, Ile^8-AII	0.21 ± 0.032	0.13 ± 0.04	
L-158,809	0.37 ± 0.036	0.32 ± 0.12	0.04 ± 0.01
L-158,338	1.4 ± 0.31	0.54	0.18 (0.04–0.8)
EXP3174	2.9 ± 1.0	1.7 ± 0.81	0.10 ± 0.02
SKF 108566	11	6.1 ± 0.25	0.54 (0.24–1.8)
DuP-753	30 ± 4.0	22 ± 2.0	5.62 (1.1–27.2)
PD-121981	>50,000	>50,000	>1000

[a] Displacement was performed in the presence of PD-121981 (0.3 μM) to occupy AT$_2$ sites; hence, only AT$_1$ binding was evaluated.

[b] K_b values with 95% confidence limits were determined by Schild plot analysis and K_b values with standard errors were determined by double-reciprocal analysis (see Materials and Methods).

TABLE 2

Displacement of specific [^{3}H]L-158,809 and ^{125}I-Sar^1, Ile^8-AII binding in rat kidney cortex and liver

Values are mean ± standard error of at least three experiments. Values without standard error were obtained from one or two experiments. The experiments were conducted and the K_i values were calculated as described for Table 1.

Displacers	K_i			
	[^{3}H]L-158,809		^{125}I-Sar^1, Ile^8-AII	
	Kidney	Liver	Kidney	Liver
	nM			
AII	12 ± 3.8	7.8 ± 2.3	5.0 ± 1.2	2.3 ± 0.45
AIII	66 ± 22	36 ± 7.5	15 ± 2.1	13 ± 6.1
Sar^1, Ile^8-AII	0.42 ± 0.06	0.12 ± 0.06	0.38 ± 0.10	0.31 ± 0.05
L-158,809	0.22 ± 0.05	0.53 ± 0.14	0.31 ± 0.04	0.37 ± 0.06
L-158,338	0.46 ± 0.03	0.71 ± 0.16	0.77 ± 0.25	0.55
EXP3174	1.2 ± 0.46	1.6 ± 0.22	1.6 ± 0.69	2.1
SKF 108566	2.6 ± 0.84	6.3	3.1 ± 0.66	3.8
DuP-753	18 ± 3.1	28 ± 8.1	48 ± 14	47 ± 17
PD-121981	>30,000	>3,000	>44,000	>3,000

liver, other target tissues of AII. However, when the nonspecific binding was defined using 1 μM unlabeled L-158,809, a portion of the specific binding in rat kidney (40%) and rat liver (10–20%) was insensitive to displacement by AII, Sar^1,Ile^8-AII, and SKF 108566 at concentrations up to 1 μM. The results suggest that [^{3}H]L-158,809 binds to additional site(s) unrelated to AII receptors. Indeed, saturation studies of specific [^{3}H]L-158,809 binding (using 1 μM L-158,809 to define nonspecific binding) and Scatchard analysis of [^{3}H]L-158,809 binding in rat kidney indicated two classes of binding sites with K_d values of 0.48 and 9.2 nM and B_{max} values of 28 pmol/g of tissue and 98 pmol/g of tissue for the high and low affinity sites, respectively (Fig. 5). Moreover, in the presence of 1 μM Sar^1,Ile^8-AII (to prevent binding to AT$_1$ and AT$_2$ receptors), L-158,809 and DuP-753 (MK-954) displaced [^{3}H]L-158,809 binding with IC$_{50}$ values of 7.4 ± 1.0 and 900 nM, respectively. In view of the apparent

TABLE 3

Effect of various pharmacological agents on specific [^{3}H]L-158,809 binding in rat adrenal

Values are mean ± standard error of a triplicate determination. All compounds were tested at 1 μM.

	Binding
	% of control
Control	100 ± 5.5
Phentolamine	91 ± 6.1
Propranolol	129 ± 23
Atropine	99 ± 3.0
Serotonin	91 ± 2.1
Haloperidol	106 ± 7.2
Bradykinin	100 ± 6
Substance P	101 ± 4.0
Lys^9-vasopressin	96 ± 8.4
Vasoactive intestinal peptide	98 ± 7.5
Naloxone	94 ± 5.3

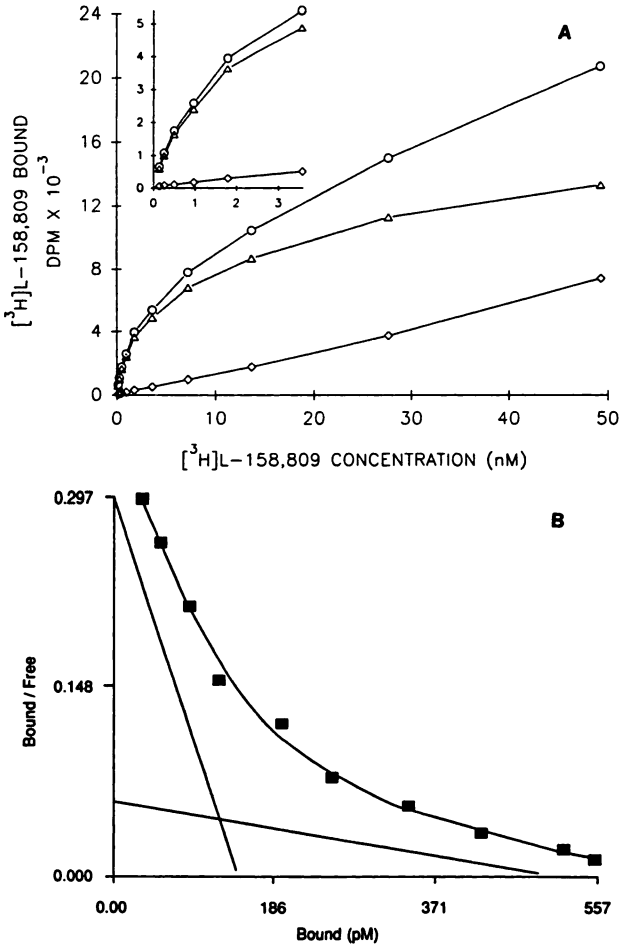

Fig. 5. [^{3}H]L-158,809 binding as a function of increasing concentrations of [^{3}H]L-158,809 in rat kidney cortex. The binding assays were performed as described in Materials and Methods, using various concentrations of [^{3}H]L-158,809. The *points* shown are means of triplicate determinations. These experiments were replicated twice with similar results. A, ○, Total binding; ◇, nonspecific binding; △, specific binding. *Inset*, enlargement showing the first six points in more detail. B, Scatchard plot of specific [^{3}H]L-158,809 binding. Data were analyzed according to the LIGAND program originally written by P. J. Munson and D. Rodbard and modified by G. A. McPherson (Elsevier-Biosoft, Cambridge, UK), assuming a two-site model.

additional binding of [^{3}H]L-158,809 to site(s) other than AII receptors in the kidney and liver, specific [^{3}H]L-158,809 binding to AII receptors in these tissues was determined using Sar^1,Ile^8-AII (1 μM) to define nonspecific binding.

The specific [^{3}H]L-158,809 binding in rat kidney and liver was inhibited by the nonpeptide AT_1 antagonists L-158,809, L-158,338, EXP3174, SKF 108566, and DuP-753 (MK-954) and the peptide antagonist Sar^1,Ile^8-AII, with K_i values comparable to their potencies in inhibiting ^{125}I-Sar^1,Ile^8-AII binding to AT_1 receptors (Table 2). AII and AIII were also effective in inhibiting the specific [^{3}H]L-158,809 binding. However, AII and AIII were 2–4 times less active in inhibiting specific [^{3}H]L-158,809 binding than inhibiting specific ^{125}I-Sar^1,Ile^8-AII binding (Table 2). PD121981 (10–100 μM), an AT_2-selective ligand, was ineffective in inhibiting [^{3}H]L-158,809 or ^{125}I-Sar^1,Ile^8-AII binding in rat kidney and liver.

Differential effect of guanyl nucleotide on the potencies of angiotensin agonists and antagonists to displace [^{3}H]L-158,809 binding in rat adrenal. The addition of Gpp(NH)p (100 μM) to the binding assay buffer had no effect on specific [^{3}H]L-158,809 binding to rat adrenal membranes. However, the K_i values of AII agonists AII and AIII in displacing specific [^{3}H]L-158,809 binding were significantly increased, by 2–3-fold (Table 4). In contrast, Gpp(NH)p had no significant effect on the K_i values of AII antagonists Sar^1,Ile^8-AII, L-158,809, and DuP-753 (MK-954). The differential effect of Gpp(NH)p on the potencies of AII agonists but not antagonists in inhibiting specific [^{3}H]L-158,809 binding further supports the finding that [^{3}H]L-158,809 binds to physiologically relevant AII receptors.

Stability of [^{3}H]L-158,809. The radiochromatograms of [^{3}H]L-158,809 incubated with adrenal, kidney, or liver membranes all exhibited a single peak, with R_F values (solvent 1, 0.58–0.63; solvent 2, 0.38–0.41) similar to those of standard [^{3}H]L-158,809. The results indicate the absence of metabolism of [^{3}H]L-158,809 during incubation in the binding assays.

Discussion

The binding of [^{3}H]L-158,809 to rat adrenal membranes was rapid, time and tissue concentration dependent, reversible, and saturable. Scatchard and Hill plot analysis indicated that [^{3}H]L-158,809 bound with high affinity (K_d = 0.66 nM) and recognized a single class of binding sites. The relative potencies (K_i) of the AII agonists AII and AIII, the AT_1-selective antagonists L-158,809 (9, 10), L-158,338 (9), EXP3174 (15), and DuP-753 (MK-954) (3, 7), and the nonselective AII antagonist Sar^1,Ile^8-AII in inhibiting specific [^{3}H]L-158,809 binding in adrenal, kidney, and liver correlated with their potencies in displacing specific ^{125}I-Sar^1,Ile^8-AII binding. The rank order of potency of the nonpeptide AII antagonists in displacing [^{3}H]L-158,809 binding also correlated with their rank order of potencies in antagonizing contractile responses to AII in the isolated rabbit aorta.

The absolute potencies of AIII in adrenal and of both AII and AIII in kidney and liver in displacing [^{3}H]L-158,809 binding appeared 2–4 times less than their potencies in displacing specific ^{125}I-Sar^1,Ile^8-AII binding. The reasons for the different K_i values for AII and AIII in inhibiting ^{125}I-Sar^1,Ile^8-AII versus [^{3}H]L-158,809 binding are not known. However, much higher tissue concentrations were used in [^{3}H]L-158,809 binding assays relative to the ^{125}I-Sar^1,Ile^8-AII binding assays, due to the lower specific activity of [^{3}H]L-158,809. The higher apparent K_i values of AII and AIII may be due to incomplete protection from degradation at the higher tissue concentrations. This contention was supported by the finding that the K_i value of AIII for displacement of ^{125}I-Sar^1,Ile^8-AII binding was increased to a value similar to its K_i in inhibiting specific [^{3}H]L-158,809 binding when higher tissue concentrations were used in the ^{125}I-Sar^1,Ile^8-AII binding assay (data not shown).

L-158,809 has been reported previously to exhibit a high selectivity for AT_1 receptors, compared with AT_2 receptors or other common peptide or nonpeptide receptors (9–11). In agreement with these reports, specific [^{3}H]L-158,809 binding in rat adrenal was not affected by AT_2-selective ligands or other pharmacological agents known to interact with various receptors.

In kidney and liver but not adrenal membranes, [^{3}H]L-158,809 appeared to bind to additional sites unrelated to AII receptors with an affinity approximately 10 times lower than for AT_1 receptors. These sites were insensitive to AII, Sar^1,Ile^8-AII, and the nonpeptide AT_1 antagonist SKF 108566 and were only sensitive to DuP-753 (MK-954) with an IC_{50} value approximately 30 times higher than for AT_1 receptors. The location and nature of the nonangiotensin [^{3}H]L-158,809 binding sites in these tissues are not known.

The ability of guanyl nucleotides to selectively affect agonist, but not antagonist, binding is well documented in several neurotransmitter receptor systems (17–20). Similarly, the addition of Gpp(NH)p reduced the affinities of AII agonists for displacing specific [^{3}H]L-158,809 binding to rat adrenal membranes. In contrast, the AII antagonists Sar^1,Ile^8-AII, L-158,809, and DuP-753 were not affected by Gpp(NH)p. The differential effects of Gpp(NH)p on displacement of [^{3}H]L-158,809 binding by AII agonists but not antagonists further support the finding that [^{3}H]L-158,809 binds to physiologically relevant AII receptors.

The use of [^{3}H]DuP-753 as an AT_1-selective radioligand has been described previously (8). The much higher (80–150-fold) affinity of L-158,809, compared with that of DuP-753, could offer some advantages of [^{3}H]L-158,809 over [^{3}H]DuP-753 as a radioligand. A high affinity ligand provides the opportunity to maximize the amount bound at a given concentration and thus provides a better ratio of specific binding to nonspecific binding. Another distinction between [^{3}H]L-158,809 and [^{3}H]DuP-753 is that [^{3}H]L-158,809 is not a prodrug, whereas [^{3}H]DuP-753 is converted to a metabolite of higher AT_1 receptor

TABLE 4

Effect of Gpp(NH)p on the K_i values of AII agonists and antagonists in inhibiting specific [^{3}H]L-158,809 binding to rat adrenal

The procedures used are as described for Table 1. Values are mean ± standard error of at least three experiments. Value without standard error was obtained from two experiments.

	K_i	
	Control	+Gpp(NH)p (100 μM)
	nM	
AII	1.7 ± 0.37	4.8 ± 1.0*
AIII	6.8 ± 0.36	13 ± 1.2*
Sar^1, Ile^8-AII	0.20 ± 0.04	0.28 ± 0.05
L-158,809	0.44 ± 0.03	0.40 ± 0.02
DuP-753	30 ± 4	35

* $p < 0.05$, compared with control.

affinity than the parent compound (15). [^{3}H]L-158,809 may thus offer advantages in studies involving the *in vivo* labeling of the AT_1 receptor.

In summary, [^{3}H]L-158,809 appears to represent a new, potent, nonpeptide, antagonist radioligand for the study of AII receptors. Its high selectivity for AT_1 as opposed to AT_2 receptors should provide a new tool for the identification of AII receptor subtypes in various tissues.

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