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The stereospecificity of LY253352 for α_1-adrenoceptor binding sites in the brain and prostate

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1 The stereospecificity of the enantiomers of LY253352, a potent and selective α_1-adrenoceptor antagonist, were studied in the human prostate and canine brain using radioligand receptor binding methods.

2 The mean equilibrium dissociation constant (K_D) in the canine brain and human prostatic adenoma was 84.4 pM and 65.4 pM, respectively.

3 The α_1-adrenoceptor density in the canine brain was approximately eight fold greater than in the human prostatic adenoma.

4 The mean K_i values of (−)-LY253352 and (+)-LY253352 in the prostate were 0.19 nM and 5.79 nM, respectively.

5 The mean K_i values of (−)-LY253352 and (+)-LY253352 in the brain were 0.29 nM and 34.7 nM, respectively.

6 This study indicates that the stereochemical specificity of the optical isomers of LY253352 is a manifestation of differential affinities of the enantiomers for α_1-adrenoceptor binding sites.

7 The differential affinities of (+)-LY253352 in the brain and prostate are suggestive of subtle unique properties of adrenoceptor binding sites in these tissues.

Introduction

The stereochemical specificity for the activation of α_1-adrenoceptors by phenethylamine derivatives has been extensively studied (for reviews see Patil *et al.*, 1974; Ruffolo, 1983). The stereochemical specificity of phenethylamine compounds with α-adrenoceptor inhibitory properties is not well recognized. LY253352 is an extremely potent and highly selective α_1-adrenoceptor antagonist with an asymmetric centre at the α-carbon atom in the phenethylamine portion of the molecule (Figure 1) (Honda *et al.*, 1985). Honda & Nakagawa (1986) have recently demonstrated that the potency of (−)-LY253352 for inhibiting phenylephrine-induced contractions in the rat prostrate is 141 times greater than (+)-LY253352. The mechanism for this isomeric activity ratio is unclear. The stereochemical specificity of LY253352 has not been previously studied in the brain.

α_1-Adrenoceptors have recently been characterized in the human and canine prostate using radioligand receptor binding methods (Lepor & Shapiro, 1984; Shapiro & Lepor, 1987). [^{125}I]-2-[-(4-hydroxyphenyl) ethylaminoethyl]tetralone ([^{125}I]-Heat) is the preferred ligand for investigating α_1-adrenoceptors in the prostate (Shapiro & Lepor, 1987). The objective of this study was to provide additional insight into the mechanism for the observed stereochemical selectivity of the enantiomers of LY253352. The relative affinity of the optical isomers of LY253352 for α_1-adrenoceptor binding sites was studied by use of radioligand receptor binding methods in the human prostate and canine brain.

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Methods

The cerebral cortex was obtained from four male beagle dogs ranging in age between 4–9 years. A craniotomy was performed following the administration of sodium pentabarbitone (25 mg kg^{-1} body weight). A segment of the cerebral cortex was excised and immediately transferred into a −70°C freezer for storage.

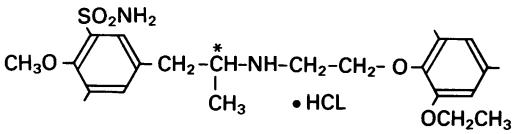

Figure 1 Chemical structure of LY253352. The asterisk indicates the asymmetric centre.

Four human prostatic adenomas were obtained at the time of surgery from men undergoing open prostatectomy secondary to symptomatic benign prostatic hyperplasia (BPH). The prostatic adenomas were obtained from men ranging in age between 55–75 years. The weight of the enucleated adenomas ranged between 50–150 g. The gross specimens were inspected by a pathologist so that areas suspicious of prostatic carcinoma were not assayed. Surgical specimens were cut into 4 mm cross-sections and representative tissues were immediately transferred into a −70°C freezer for storage. The microscopic diagnosis on all tissues submitted to pathology was BPH.

Preparation of tissue homogenates

The brain and prostate specimens were weighed, immersed directly in liquid nitrogen, and pulverized using a Thermovac tissue pulverizer. The tissue fragments were homogenized in 20 vol (v/w) of ice-cold 5 mM potassium phosphate buffer (pH 7.4) with a Brinkman polytron at a speed setting of 6 for 60 s. The homogenates were centrifuged at 29,000 *g* in a Sorvall RC-5B centrifuge using a Sorval SS-34 rotor for 10 min at 4°C. The pellets were homogenized in 20 vol (v/w) of Krebs-Ringer-Phosphate buffer (pH 7.4) containing 100 μM NaGTP. The homogenates were shaken on a New Brunswick G2 gyrotory shaker at room temperature for 30 min at a speed setting of 100 r.p.m. and then centrifuged at 29,000 *g* for 10 min at 4°C. The pellets were washed twice with 20 vol (v/w) of ice-cold 50 mM sodium potassium phosphate buffer (pH 7.4). The pellets were homogenized and centrifuged at 29,000 *g* for 10 min at 4°C between washes. The final pellets were homogenized in 50 vol (v/w) of 50 mM sodium potassium phosphate buffer (pH 7.4) at room temperature and filtered through fine Nitex mesh gauze.

Saturation analysis

Saturation analyses were performed using 7 different concentrations (0.01 nM to 0.40 nM) of [^{125}I]-HEAT (2-[-(4-hydroxyphenyl)ethylaminoethyl]tetralone) at constant specific activity (2200 Ci mmol^{-1}). Total binding was determined in 500 μl containing 400 μl tissue homogenate, 50 μl of 50 mM sodium potassium phosphate buffer (pH 7.4), and 50 μl [^{125}I]-HEAT at various concentrations. Total and non-specific binding determinations for each [^{125}]-HEAT concentration were performed in triplicate in polypropylene test tubes. The assay tubes were shaken on a New Brunswick G2 gyrotory shaker for 30 min at room temperature. Maximal specific binding was consistently observed in brain and prostatic homogenates at a HEAT concentration of 80 pM following an incubation interval of 30 min (data not shown). The binding assays were terminated by filtering over Whatman 2.4 cm GF/B glass fibre filters placed in a 45 well vacuum manifold. The glass filter discs were washed 4 times with 4 ml of ice cold 50 mM sodium potassium phosphate buffer (pH 7.4) containing 10% w/v polyethylene glycol (molecular weight approximately 3350) under vacuum suction. The glass filter discs were placed in 10 ml of aqueous accepting scintillation fluid (BudgetSolve, RPI, Mount Prospect, Il) and the vials counted on a LS-3801 liquid scintillation counter (Smith, Kline, Beckman, Fullerton, Ca) at a calculated average efficiency of 63%. Saturation curves, Scatchard plots, K_D, B_{max}, and the linear correlation coefficients of the observed Scatchard plots were generated by the computer programmes described below. Specific [^{125}I]-HEAT binding accounted for 50% of total [^{125}I]-HEAT binding in the human prostate at a [^{125}I]-HEAT concentration of 80 pM.

Competitive binding experiments

Competitive binding experiments were carried out in the presence of a constant concentration of [^{125}I]-HEAT and increasing concentrations of unlabelled (−)-LY253352 and (+)-LY253352. Tissue homogenates were prepared in the manner previously outlined. Four hundred μl of tissue homogenate, 50 μl of 80 pM [^{125}I]-HEAT, and 50 μl of the various concentrations of the unlabelled optical isomers of LY253352 were combined and shaken at 100 r.p.m. on a New Brunswick G2 gyrotory shaker at room temperature for 30 min. The assays were repeated in quadruplicate for each concentration of inhibitor. The assays were terminated as previously described. Four separate competitive displacement experiments were performed on prostatic and brain homogenates for each of the optical isomers. Non-specific binding represented [^{125}I]-HEAT bound in the presence of 10^{-5} M phentolamine.

Specific binding was determined by subtracting the non-specific binding component from the total [^{125}I]-HEAT binding at the various inhibitor concentrations. Maximal binding was determined in the presence of only [^{125}I]-HEAT. A [^{125}I]-HEAT concentration of 80 pM was used in all competitive binding assays. The IC_{50} represents the concentra-

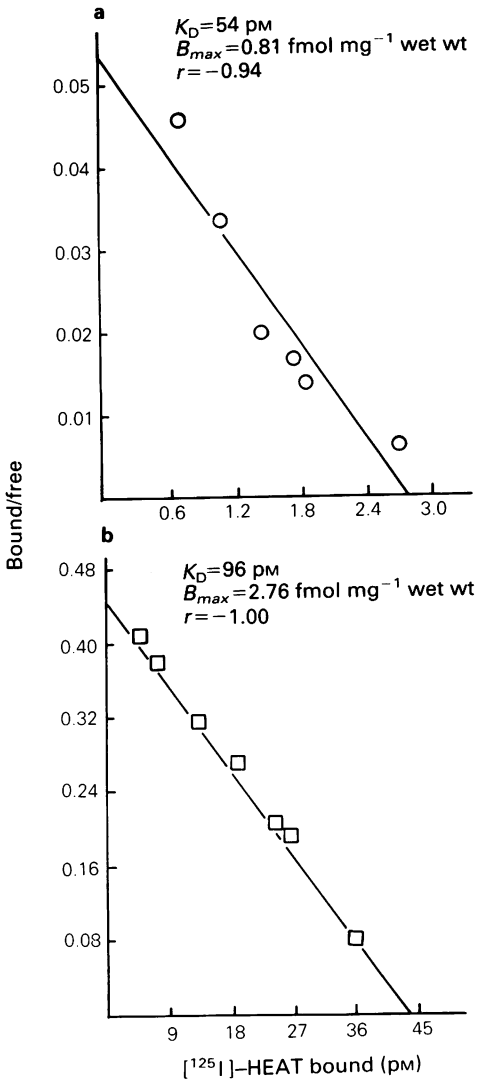

Figure 2 Representative Scatchard plots for [^{125}I]-HEAT binding in homogenates of human prostatic adenoma removed by open prostatectomy (a) and dog cerebral cortex (b). The equilibrium dissociation constant (K_D), receptor concentration (B_{max}), and correlation coefficient (r) were determined from the Scatchard plots using the computer programme SCAFIT.

tion of unlabelled α-adrenoceptor antagonist inhibiting 50% of the maximum [^{125}I]-HEAT specific binding. IC_{50} values were graphically determined from the plot of −log optical isomer concentration vs % maximal [^{125}I]-HEAT specific binding. Competition data were analysed using the BASIC programme EBDA (McPherson, 1983) which provides initial parameter estimates and formats the data for analysis by the programme SCAFIT (Munson & Rodbard, 1980).

Table 1 [^{125}I]-HEAT binding in human prostate adenoma and canine brain

Tissue	*Assays*	K_D (pM)	B_{max} (fmol mg^{-1} wet wt)
Human prostate	4	65.4 ± 19.2	0.37 ± 0.15
Canine brain	4	84.4 ± 4.3	2.88 ± 0.10

Four saturation experiments were performed on tissue homogenates obtained from human prostatic adenomas and canine cerebral cortex. The equilibrium dissociation constant (K_D) and receptor concentration (B_{max}) were determined from Scatchard plots using the computer programme SCAFIT. The data are expressed as mean ± s.e.mean.

Materials

[^{125}I]-HEAT (specific activity 2200 Ci mmol^{-1}) was obtained from New England Nuclear Corporation. Other compounds and their sources were: phentolamine (Regitine HCl, Ciba-Geigy Corporation, Summit, NJ); polyethylene glycol (Sigma Chemical Co., St. Louis, MO); (−)-LY253352 and (+)-LY253352 were a generous gift from the Eli Lilly Company (Indianapolis, In).

Results

Saturation experiments were performed using tissue homogenates obtained from four canine brains and four human prostatic adenomas. Representative Scatchard plots of [^{125}I]-HEAT binding in homogenates obtained from the canine brain and human prostatic homogenates are shown in Figure 2. The binding of [^{125}I]-HEAT in the canine brains and human prostatic adenomas was consistently saturable and of high affinity. The Scatchard plots were linear, suggesting a single class of [^{125}I]-HEAT binding sites. The equilibrium dissociation constants (K_D) for [^{125}I]-HEAT binding in the canine brains and human prostatic adenomas were 84.4 ± 4.3 pM and 65.4 ± 19.2 pM, respectively (mean ± s.e.mean), (Table 1). The similar equilibrium dissociation constant for these tissues ($P > 0.05$) is indicative of homogeneity of α_1-adrenoceptor binding sites. The α_1-adrenoceptor concentrations in the canine brains

Table 2 Competitive displacement of [^{125}I]-HEAT binding in human prostatic adenoma and canine brain by the optical isomers of LY253352

Tissue	Assays	K_i (IC_{50} corr.) (nM) (−)-LY253351	(+)-LY267592
Human prostate	4	0.19 ± 0.11	5.79 ± 0.67
Canine brain	4	0.29 ± 0.04	34.7 ± 5.0

Competitive binding experiments were performed using 80 pM [^{125}I]-HEAT and various concentrations of the unlabelled optical isomers of LY253352 in 4 separate homogenates obtained from human prostatic adenomas and canine cerebral cortex. Competitive displacement data were analysed using the BASIC programme EBDA (McPherson, 1983) which provides initial parameter estimates and formats the data for analysis by the programme SCAFIT (Munson & Rodbard, 1980). The K_i values for the optical isomer are expressed as mean ± s.e.mean.

and human prostatic adenoma were 0.37 ± 0.15 fmol mg^{-1} wet wt and 2.88 ± 0.10 fmol mg^{-1} wet wt, respectively (mean ± s.e.mean) (Table 1).

The IC_{50} and the K_i (IC_{50} corr.) for the displacement of specific [^{125}I]-HEAT binding by (−)-LY253352 and (+)-LY253352 were determined using competitive binding assays. Four separate competitive binding assays were performed in quadruplet for each enantiomer of LY253352 in homogenates obtained from canine brains and human prostatic adenomas. A computer-generated plot for specific [^{125}I]-HEAT binding vs −log of the concentration of the optical isomers of LY253352 is shown in Figure 3. The K_is for the optical isomers were determined using the computer programme analysis of the competitive displacement data (Munson & Rodbard, 1980; McPherson, 1983) (Table 2). The K_is for (−)-LY253352 and (+)-LY253352 in the canine brain were 0.29 ± 0.04 nM and 34.7 ± 5.0 nM, respectively (mean ± s.e.mean) (Table 2). The affinity of (−)-LY253352 for α_1-adrenoceptor binding sites in the canine brain was 120 times greater than the affinity of (+)-LY253352. The K_is for (−)-LY253352 and (+)-LY253352 in the human prostatic adenoma were 0.19 ± 0.11 and 5.79 ± 0.67 nM, respectively (Table 2). The affinity of (−)-LY253352 for the α_1-adrenoceptor binding sites in human prostatic adenoma was 30 times greater than the affinity of (+)-LY253352. The K_is of (−)-LY253352 in the canine brain and human prostate adenoma were similar ($P > 0.05$). The K_is of (+)-LY253352 in the canine brain and human prostate were different

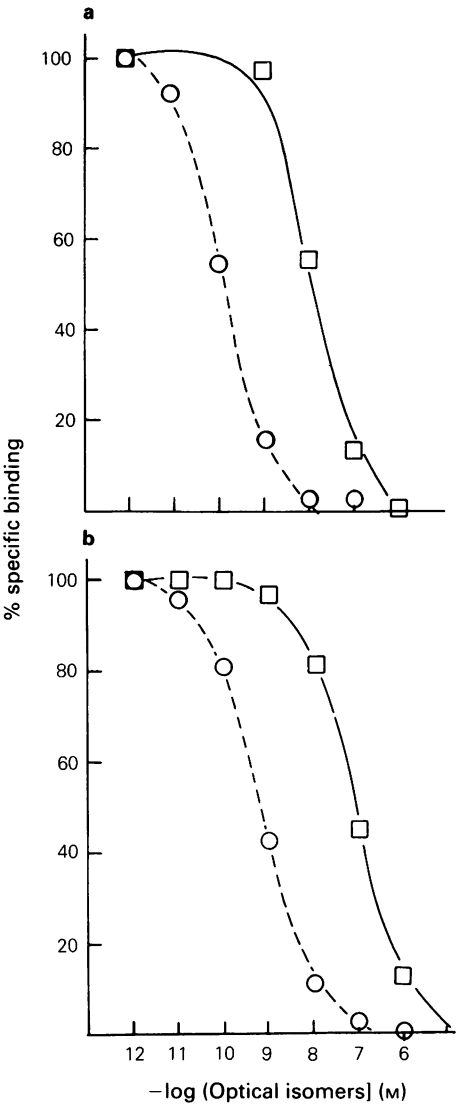

Figure 3 Competitive binding assays were performed using 80 pmol [^{125}I]-HEAT and varying concentrations of the unlabelled optical isomers of LY253352 in tissue homogenates obtained from human prostatic adenoma and dog cerebral cortex. The competitive displacement data were analysed using the BASIC programme EBDA (McPherson, 1983) which provides initial parameter estimates and formats the data for analysis by the programme SCAFIT (Munson & Rodbard, 1980). A representative competitive binding experiment is illustrated for a tissue homogenate of human prostatic adenoma (a) and dog cerebral cortex (b). The competitive displacement curve for (−)-LY253352 is represented by (○---○) and (+)-LY253352 by the solid lines (□——□).

($P < 0.05$) suggesting that there is some heterogeneity of the α_1-adrenoceptor binding sites in these tissues.

Discussion

α_1-Adrenoceptors have not been extensively studied in either urogenital or male accessory sex tissues using radioligand receptor binding methods owing to excessive non-specific binding (Caine, 1983). α_1-Adrenoceptors have recently been characterized in human prostatic adenomas (Lepor & Shapiro, 1985) and canine prostates (Shapiro & Lepor, 1987) using [^{3}H]-prazosin and [^{125}I]-HEAT, respectively. In this study, [^{125}I]-HEAT binding in human prostatic adenomas was saturable and the Scatchard plots were linear, indicating a single population of binding sites. The mean equilibrium dissociation constant for [^{125}I]-HEAT binding in human prostatic adenomas was similar to values obtained by other investigators in the brain and peripheral tissues (Minneman *et al.*, 1983, Sugden & Klein, 1984). This study represents the first comparative analysis of α_1-adrenoceptor binding in the prostate and brain. There was no statistically significant difference between the mean K_Ds in these tissues. The α_1-adrenoceptor density in the human prostatic adenomas determined in the present study (0.37 fmol mg^{-1} wet wt) was similar to values obtained in human prostatic adenomas using [^{3}H]-prazosin (1.12 fmol mg^{-1} wet wt). The similar receptor density obtained is further evidence that these ligands measure α_1-adrenoceptor density selectively. In this study the α_1-adrenoceptor density in the canine brain was eight fold greater than in the human prostate.

Honda & Nakagawa (1986) recently demonstrated that the competitive antagonism of (−)-LY253352 and (+)-LY253352 against phenylephrine-induced contractions in the rabbit trigone, urethra, and prostate is stereoselective. The observed pA_2 values for (−)-LY253352 and (+)-LY253352 in the prostate were 9.92 (1.2×10^{-10}) and 7.77 (1.7 ± 10^{-9}), respectively. The present study confirmed the stereochemical specificity of the optical isomers of LY253352 in human prostatic adenomas using competitive binding assays. The mean K_is for the displacement of specific [^{125}I]-HEAT binding by (−)-LY253352 and (+)-LY253352 were 0.19 nM and 5.79 nM, respectively. The observed affinities of the optical isomers of LY253352 for the inhibition of phenylphrine- induced contractions in the rabbit prostate and the displacement of [^{125}I]-HEAT specific binding in the human prostate are similar, implying a direct relationship between receptor binding and muscle contractile activity. The competitive antagonism of phenylephrine-induced contractions was 141 times more potent in the rabbit prostate for (−)-LY253352 compared to (+)-LY253352. The present study indicates that the stereochemical specificity of the optical isomers of LY253352 is a manifestation of the relative affinities of the enantiomers for the α_1-adrenoceptors.

The stereochemical specificity of the optical isomers of LY253352 has not been previously evaluated in the brain. The present study demonstrated that the binding of the optical isomers of LY253352 in the canine brain is also stereospecific. The K_is for the displacement of specific [^{125}I]-HEAT binding by (−)-LY253352 and (+)-LY253352 were 0.29 nM and 34.7 nM, respectively. The isomeric ratio for the affinities of the enantiomers of LY253352 for [^{125}I]-HEAT binding in the canine brain was 120. There was no significant difference between the K_is for LY253352 in the brain and prostate. The K_is of (+)-LY253352 in the brain and prostate were significantly different ($P < 0.05$). The differential affinities of (+)-LY253352 in the brain and prostate are suggestive of subtle unique properties of α_1-adrenoceptor binding sites in these tissues. The observations in this study may have significant clinical relevance. Several recent clinical trials have demonstrated that obstructive urinary symptoms in men with benign prostatic hyperplasia are improved following the administration of α_1-adrenoceptor antagonist drugs (Caine *et al.*, 1978; Hedlund *et al.*, 1983). The *in vitro* contractile properties of prostatic tissues are mediated by α-adrenoceptors (Honda *et al.*, 1985). The observed clinical efficacy of α_1-adrenoceptor antagonists in men with symptomatic benign prostatic hyperplasia is presumably related to a drug-mediated relaxation of the smooth muscle component of the prostate adenoma. The binding of α_1-adrenoceptor antagonists to the smooth muscle of the prostate probably results in the diminution of prostatic urethral resistance, thereby facilitating micturition. Transurethral resection of the prostate currently represents the accepted treatment for symptomatic benign prostatic hyperplasia. There is increasing interest for treating symptomatic benign prostatic hyperplasia with α_1-adrenoceptor antagonists. LY253352 is a selective α_1-adrenoceptor antagonist that is more potent than prazosin. The treatment of symptomatic benign prostatic hyperplasia with any new drug containing an asymmetric carbon must take into consideration the potential for stereochemical selectivity of the optical isomers.

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