

Pharmacologic Characterization of CHIR 2279, an N-Substituted Glycine Peptoid with High-Affinity Binding for α_1 -Adrenoceptors

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ABSTRACT

We characterize the *in vitro* and *in vivo* pharmacology of CHIR 2279, an N-substituted glycine peptoid previously identified from a combinatorial library as a novel ligand to α_1 -adrenoceptors. Competitive receptor-binding assays with [3 H]prazosin showed that CHIR 2279 was similar to prazosin in binding to α_{1A} (rat submaxillary), α_{1a} , α_{1b} and α_{1c} (cDNA expressed in LTK⁻ cells) with high and approximately equipotent affinity. K_i values for CHIR 2279 ranged from 0.7 to 3 nM, and were 10-fold weaker than with prazosin. Functional assays for postsynaptic α_1 -adrenoceptors showed CHIR 2279 was approximately equipotent in antagonizing agonist-induced contractile responses with rat vas deferens (α_{1A}), canine prostate (α_{1A}), rat spleen (α_{1B}) and rat aorta (α_{1D}). The pA_2 for CHIR 2279

averaged 7.07 in these assays, indicating a 10- to 100-fold lower *in vitro* potency than prazosin. In dogs, CHIR 2279 antagonized the epinephrine-induced increase in intraurethral pressure (pseudo pA_2 , 6.86) and in rats antagonized the phenylephrine-induced increase in mean arterial blood pressure. In rats and guinea pigs, CHIR 2279 induced a dose-dependent decrease in mean arterial blood pressure without eliciting the tachycardia commonly observed with other α_1 -blockers. Pharmacokinetic/pharmacodynamic modeling showed the i.v. systemic clearance rate of CHIR 2279 was 60 and 104 ml/min/kg in rats and guinea pigs, respectively, and the *in vivo* potency for mean arterial blood pressure reduction was twice as great in guinea pigs (EC_{50} , 520 ng/ml) than rats (EC_{50} , 1170 ng/ml).

A competitive advantage for the discovery of new lead structures has been afforded to pharmaceutical companies that have amassed large collections of compounds. These collections have included molecules accumulated over many years of synthetic efforts as well as cumulations of natural products derived from plants, fermentations, marine organisms and insect toxins (Hylands and Nesbit, 1991). Recent advances in both the biological synthesis of peptides (Bull *et al.*, 1993) and the solid-phase synthesis of multiple peptides

(Jung and Beck-Sickinger, 1992; Geysen *et al.*, 1984; Houghten, 1985) have led to the generation of peptide libraries with millions of compounds. Solid-phase organic synthesis of nonnatural polymers and heterocyclic compounds has extended the diversity of chemical libraries (Desai *et al.*, 1994; Hobbs DeWitt, 1994; Pavia *et al.*, 1993; Gallop *et al.*, 1994; Gordon *et al.*, 1994).

Recently, the generation and screening of complex chemical libraries has emerged as an important strategy for the identification of therapeutic leads (Desai *et al.*, 1994). Advances in molecular biology and automation have resulted in

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ABBREVIATIONS: CHIR 2279, ([N-(2-(4-hydroxyphenyl)ethyl)glycyl]-[N-((4-phenyl)phenyl)glycyl]-N-(2-(phenyl)ethyl)glycinamide); 5HT, serotonin; CL, systemic clearance rate; DA, dopamine; DMEM, Dulbecco's modified Eagle's medium; EC_{50} , the concentration in the effect compartment at which 50% of the maximum pharmacologic effect is observed; EDTA, ethylenediamine-tetraacetic acid; E_{max} , maximum pharmacologic effect observed; γ , the sigmoidicity parameter; HPLC, high performance liquid chromatography; IUP, intraurethral pressure; K_{10} , the first-order rate constant for elimination from the central compartment; K_{e0} , the first-order distribution rate constant for transfer from the central to the effect compartment; K_{12} , the first-order distribution rate constant for transfer from the central to the peripheral compartment; K_{21} , the first-order distribution rate constant for transfer from the peripheral to the central compartment; MAP, mean arterial blood pressure; NE, norepinephrine; NSG, N-substituted glycine peptoid; PBS, phosphate-buffered saline; PK/PD, pharmacokinetic/pharmacodynamic; $T_{1/2\alpha}$, half-life corresponding to the first α elimination phase; $T_{1/2\beta}$, the half-life corresponding to the second β elimination phase; $T_{1/2Ke0}$, the half-life corresponding to transfer from the central compartment to the effect compartment; Tris, tris(hydroxymethyl)-aminomethane; V_c , initial volume of distribution; V_β , volume of distribution during the β -phase of elimination; WB- 4101, 2(N[2,6-di-methoxyphenoxy-ethyl]) amino-methyl-1,1-benzodioxane.

rapid, high-throughput biological assays. As a consequence of these advancements, significant strides have been made in the acceleration of therapeutic lead discovery.

Oligomeric N-substituted glycines represent the first application of a modular approach to the synthesis of nonnatural chemical libraries (Simon *et al.*, 1992). Libraries of NSG are desirable for several reasons: 1) the molecules contain a wide variety of functional groups; 2) the monomers are easily synthesized through a highly efficient route that is robotically automated (Zuckermann *et al.*, 1992), providing for the incorporation of thousands of primary amines that are readily available as building blocks and 3) the resulting compounds are stable to hydrolytic enzymes (Miller *et al.*, 1994).

An example of the use of an NSG library for accelerated lead discovery was recently reported by Zuckermann and coworkers (1994). These researchers generated a diverse, *ca.* 5000-component library that was designed to contain likely ligands to receptors in the family of 7-transmembrane G-protein-coupled receptors. The NSG peptoids¹ in the library were screened in solution-phase, competitive receptor-binding assays, leading to the first discovery of high-affinity receptor ligands from a combinatorial library of nonnatural chemical entities. One of the ligands identified in this work, CHIR 2279 was shown to have a K_i of 5 nM for α_1 -adrenoceptors in an assay with [³H]prazosin in rat fore-brain tissue.

In our investigation we characterized the pharmacology of the novel α_1 -adrenoceptor antagonist, CHIR 2279. Our objective was to determine: 1) the specific subclasses of α_1 -adrenoceptors to which CHIR 2279 binds with high affinity; 2) the functional activity of CHIR 2279, as defined by standard *in vitro* assays for adrenoceptor ligands and 3) the *in vivo* activity of CHIR 2279 in animal models that test for effects mediated by α_1 -adrenoceptors.

Materials and Methods

CHIR 2279. CHIR 2279 is a trimeric NSG peptoid with a molecular mass of 569 g/mol (fig. 1). Synthesis of CHIR 2279 was performed as described previously (Zuckermann *et al.*, 1994) and purification was implemented by reverse-phase HPLC. Before use in the experiments, CHIR 2279 was shown by analytical HPLC and mass spectrometry to be >99% pure.

Adrenoceptor nomenclature. The nomenclature for α_1 -adrenoceptors in this report was based on a recently published scheme for adrenoceptor classification (Hieble *et al.*, 1995).

Creation of cell lines. The cDNA clones encoding α_1 -adrenoceptors were provided by Triangle Universities Licensing Consortium (Research Triangle Park, NC) and were inserted into the eukaryotic expression vector SnB30. The sources of the clones were as follows: α_{1a} , cow; α_{1b} , hamster and α_{1d} , rat. Expression of the receptor gene in the vector was under the transcriptional control of an SV40 early promoter and positive drug selection was provided by a gene for neomycin resistance. Mouse fibroblast cells (LTK⁻) were transfected with the α_1 expression plasmids and grown in DMEM containing 10% fetal calf serum and 30 μ M geneticin® (Gibco BRL Life Technologies, Gaithersburg, MD). Stable cell lines were generated that were resistant to geneticin, and expression of the receptor proteins

¹ Oligomeric N-substituted glycines are referred to as "peptoids" in recognition of their conceptual lineage. These molecules are isomers of peptides in which the glycine backbone is maintained, but the sidechains attach at the amide nitrogen.

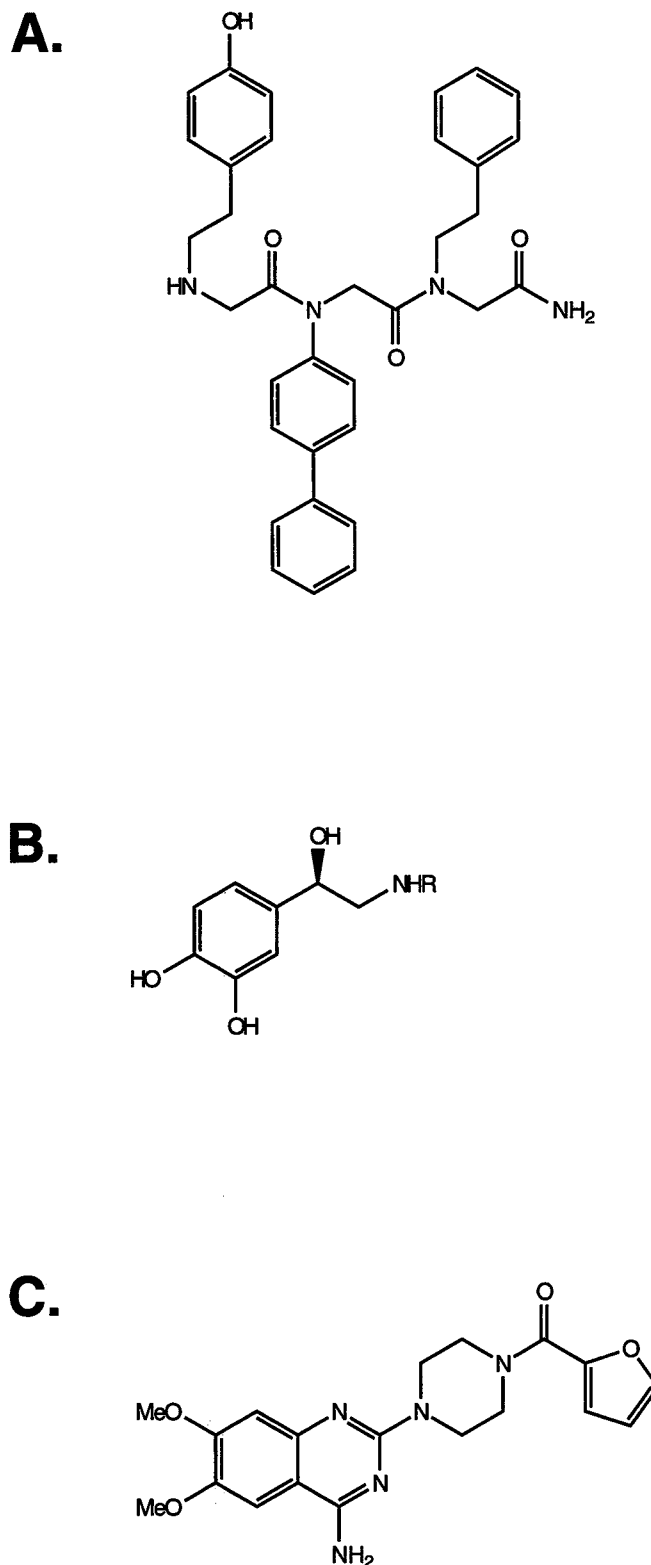


Fig. 1. A, Chemical structure of CHIR 2279. B, Chemical structure of norepinephrine (R = H) and epinephrine (R = Me). C, Chemical structure of prazosin.

was monitored by radioligand binding. Stable clones of single cells were derived from the parental lines and were screened in receptor binding assays to identify clones with high densities of receptors. Roller bottle cultures of the cloned lines were used to provide cell

membranes for quantitation of receptor binding. A cell line that contained the SnB30 vector with the human gene for erythropoietin was used as a control in the binding assays.

Tissue culture of transfected cells and membrane preparation. For receptor binding assays, large scale preparations of membranes were used in which 6×10^6 cells were seeded into 450-cm² roller bottles for tissue culture (Corning Inc., Corning, NY). A 200-ml volume of DMEM amended with 10% fetal calf serum and 300 μ M geneticin was added to each roller bottle and a sterile gas mixture of 95% air and 5% CO₂ was injected before sealing. The bottles were incubated at 37°C on a roller rack for 5 days and fresh medium was provided after 3 days in culture. On the fifth day of culture, the growth medium was removed from the roller bottles and the cells were washed twice with PBS (Sigma Chemical Co., St. Louis, MO; 120 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄-NaH₂PO₄, pH 7.4).

To detach the cells from the roller bottles, a 15-min incubation was performed at 37°C with a solution containing Tris-EDTA (10 mM Tris, 100 mM NaCl, 1 mM EDTA, pH 7.4). The cell suspension was decanted into tared centrifuge tubes placed on ice and an aliquot was taken for cell counting. The cells were centrifuged at 3000 \times g for 5 min at 2–4°C, washed with PBS, centrifuged and the pellets were weighed to determine the wet weight of the cells. The cells were washed a final time in 5 mM Tris-HCl, 5 mM EDTA, pH 7.7 (40 volumes per gram wet weight); centrifugations after this and subsequent procedures were performed at 40,000 \times g for 10 min. The cells were homogenized in 50 mM Tris-HCl, 5 mM EDTA (pH 7.4) and then centrifuged. The pellets were rehomogenized in 50 mM Tris-HCl (pH 7.4), centrifuged and the final pellets were resuspended in 6.25 volumes (per gram wet weight) of 50 mM Tris-HCl. Aliquots of the pooled homogenates were frozen in liquid nitrogen and stored at -70°C until the time of assay. Rat submaxillary glands were used for α_{1A} receptors and were prepared as described (Michel *et al.*, 1989).

Radioligand binding assays. Receptor binding assays for α_1 -adrenoceptors were performed as described by Greengrass and Bremner (1979). Briefly, a buffered solution of 50 mM Tris-HCl (pH 7.7) was used to dilute the membrane homogenates from the cloned receptors 96-fold and to dilute the homogenates from submaxillary glands 12-fold. Plastic Bioblocks (DBM Scientific, Valencia, CA) were incubated at 25°C for 50 min with 500 μ L of diluted membrane homogenates, 450 μ L of [³H]prazosin (0.2 nM final concentration; 75–85 Ci/mMol, Du Pont-NEN Corp., Boston, MA) and 50 μ L of either water (for total binding) or 10 μ M phentolamine (final concentration, for nonspecific binding). After equilibration, bound radioligand was separated from free radioligand with GF/B filters presoaked in 0.5% polyethyleneimine with either a Brandel (Brandel, Inc., Gaithersburg, MD) or a Packard (Packard Instruments Co., Downer's Grove, IL) cell harvester. Radioactivity was determined by standard techniques for liquid scintillation counting and data were analyzed as described by Cheng and Prusoff (1973).

Receptor binding assays for subtypes of α_2 -adrenoceptors were performed as described elsewhere (Giardina *et al.*, 1993; Hancock *et al.*, 1995). Briefly, human clonal α_{2A} and α_{2C} adrenoceptors (Triangle Universities Licensing Consortium) expressed in LTK-cells and α_{2B} receptors from neonatal rat lung membranes were incubated in a 1-ml final volume of 25 mM glycylglycine buffer (pH 7.4). The buffer was amended with 500 μ L of membrane homogenates, 450 μ L of [³H]rauwolscine (0.2 nM final concentration; 75–85 Ci/mmol, Du Pont-NEN Corp., Boston, MA) and 50 μ L of either water (for total binding) or 10 μ M phentolamine (final concentration, for nonspecific binding) or test agent and the incubations were implemented at 0°C for 120 min.

Receptor binding assays for β_1 and β_2 receptors were performed essentially as described by U'Prichard *et al.* (1978) with previously reported modifications (Giardina *et al.*, 1993). Rat ventricle (β_1) or lung (β_2) tissues were incubated in a 1-ml final volume of 50 mM Tris-HCl (pH 7.7) consisting of 500 μ L of membrane homogenates,

450 μ L of [³H]dihydroalprenolol (0.2 nM final concentration; 90–100 Ci/mmol, Du Pont-NEN Corp.) and 50 μ L of either water (for total binding) or 1 μ M propranolol (final concentration, for nonspecific binding) or test agent. The incubations were carried out at 25°C for either 30 min (β_1) or 50 min (β_2).

Receptor binding assays for 5HT₁ receptors were performed as described by Pedigo *et al.* (1981). Rat cortex was incubated in a 1-ml final volume of 50 mM Tris-HCl (pH 7.4) containing 4 mM CaCl₂, 5.7 mM ascorbic acid and 10 μ M pargyline. This solution was amended with 500 μ L of membrane homogenate, 450 μ L of [³H]5HT (0.2 nM final concentration; 25–30 Ci/mmol, Du Pont-NEN Corp.) and 50 μ L of either water (for total binding) or 10 μ M 5HT (final concentration, for nonspecific binding) or test agent. The incubations were performed at 37°C for 15 min.

Receptor binding assays for 5HT₂ receptors were performed as described by Leysen *et al.* (1982). Rat cortex was incubated in a 1-ml final volume of 50 mM Tris-HCl (pH 7.7) containing 500 μ L of membrane homogenate, 450 μ L of [³H]ketanserin (0.4 nM final concentration; 60–70 Ci/mmol, Du Pont-NEN Corp.) and 50 μ L of either water (for total binding) or 1 μ M methysergide (final concentration, for nonspecific binding) or test agent and incubations were carried out at 37°C for 15 min.

Data for all radioligand binding experiments were analyzed as previously reported (Hancock *et al.*, 1988) and K_i values were determined as described by Cheng and Prusoff (1973).

Assays for inhibition of uptake of biogenic amines. Experiments were conducted to determine the potency of CHIR 2279 as an inhibitor of the uptake of biogenic amines. As previously described by Hancock *et al.* (1995), synaptosomes were prepared from freshly dissected sections of rat hypothalamus, cortex and striatum for studies of [³H]-NE (12–15 Ci/mmol), [³H]-5HT (25–30 Ci/mmol) or [³H]-DA (25–30 Ci/mmol) uptake, respectively. Tissue was obtained from Sprague-Dawley rats of either sex. Radiolabeled amines were obtained from Du Pont-NEN Corp. Tissues were homogenized in 10-, 5- or 20-volumes, respectively, of 0.32 M sucrose, using a Potter-Elvehjem tissue grinder. These homogenates were centrifuged at 1000 \times g for 10 min and the supernatant fractions harvested for use in the assay. Aliquots of tissue (100 μ L) were added to 750 μ L of Krebs solution (composition in mM: NaCl 118, KCl 4.0, CaCl₂ 1.13, KH₂PO₄ 1.12, MgSO₄ 1.20, NaHCO₃ 2.4, D-glucose 5.0, Na₂EDTA 1.5, ascorbic acid 1.0 and pargyline 0.0125; pH adjusted to 7.4 after aeration with 95% O₂, 5% CO₂), 50 μ L of test compound and 100 μ L of [³H]-amine (final concentration, approximately 100 nM). Tissues were incubated for 4 min at 37°C, followed by rapid vacuum filtration as described for the receptor binding assays. Nonspecific uptake and/or binding was determined in tubes containing 50 μ L water instead of compound and incubated for 4 min at 0°C. Data were analyzed as described above to determine IC₅₀ values for inhibition of amine uptake by the test compounds.

Isolated tissue bioassays. Contractions mediated by α_1 -adrenoceptors were examined in slices obtained from rat vas deferens, rat spleens, rat aorta and canine prostate glands. Whole rat vas deferens, spleen and aorta were studied according to the procedures of Aboud *et al.* (1993), with the exception that phenylephrine, rather than NE, was used as the agonist. Canine prostate glands were obtained from male beagle dogs that were at least 2 yr in age. Slices (3–5 mm) were cut parallel to the plane of the urethra and mounted in isolated tissue chambers as described for the vas deferens (Aboud *et al.*, 1993) in a Krebs-Henseleit buffer containing 119 mM NaCl, 4.7 mM KCl, 1.2 mM KH₂PO₄, 2.5 mM CaCl₂, 0.01 mM K₂EDTA, 20 mM NaHCO₃, 1.5 mM Mg₂SO₄, 11 mM dextrose and 0.004 mM propranolol. Concentration-response curves for the agonist were generated in the presence and absence of test compounds and the data were analyzed as described by Schild (1947). For all of the bioassays, each antagonist was tested with a separate tissue.

Stability and solubility assessments for CHIR 2279. To confirm that CHIR 2279 was soluble under the various *in vitro* assay conditions used, the peptoid was added directly to the buffers used in

the assays at each of the two highest concentrations used in the specific assay. The media were then incubated under conditions used during the assay. For example, for the *in vitro* functional assays, CHIR 2279 was examined in Kreb's Henseleit buffer at 1 and 3 μM after incubation at 37°C for 1 hr. After the incubations, aliquots were sampled and measured for CHIR 2279 by reverse phase HPLC. Inasmuch as CHIR 2279 was previously found to have good solubility in 2% acetonitrile/water, this medium was used as a reference. Comparisons of HPLC peak areas (CHIR 2279 in assay buffer vs. CHIR 2279 in 2% acetonitrile/water) indicated that CHIR 2279 was soluble under the various *in vitro* assay conditions used in this report.

To demonstrate that CHIR 2279 was not generally susceptible to metabolic degradation by isolated tissues, two tests were performed. First, ^{14}C -CHIR 2279 (30 Ci/mol; Chiron Corp., Emeryville, CA) and CHIR 2279 at 0.3 μM were added to tissue baths containing isolated tissues, and conditions were established as in typical functional assays. Aliquots of the media in the tissue baths were sampled immediately after addition of the CHIR 2279 and at 30-min intervals up to 2 hr. That degradation of CHIR 2279 did not occur during these incubations was confirmed by reverse phase HPLC: since the ^{14}C label did not appear on chemical species other than the ^{14}C -labeled parent compound (including an analysis of the void volume), it was deduced that CHIR 2279 was not vulnerable to metabolic degradation by tissues used in the functional assays. A second assay to determine the general metabolic stability of CHIR 2279 was performed with homogenates of rat small intestine. This assay showed that 95% of the parent compound remained intact after a 90-min incubation at 37°C, suggesting that CHIR 2279 is generally invulnerable to degradation by the peptidases and other hydrolytic enzymes that abound in the gut².

IUP in dogs. Male beagle dogs (Marshall Farms, NorthRose, NY) that were 2 or more yr in age and weighed between 12 and 15 kg were used to measure the effects of α -adrenergic agonists and antagonists on prostatic urethral tone. To record IUP, a balloon catheter was inserted into the prostatic urethra through the penis of fully anesthetized dogs (Brune *et al.*, 1995). Various doses of the antagonist were administered (0, 0.3, 1 and 3 mg/kg), followed after 15 min with epinephrine (0.01–300 $\mu\text{g}/\text{kg}$). The maximal change in IUP elicited by epinephrine was approximately 50 mm Hg. Generation of epinephrine dose-response curves, experimental design and statistical analysis of the data were performed as described by Brune *et al.* (1995). The antagonists were administered by i.v. bolus in a vehicle of 25% hydroxy- β -cyclodextrin (Sigma Chemical Co., St. Louis, MO) in water, in a dosing volume of 0.1 ml/kg body weight.

MAP, heart rate and pharmacokinetics. Male CD rats (0.3–0.5 kg; Charles River, Wilmington, MA) and Duncan-Hartly guinea pigs (0.3–0.5 kg; Charles River) were used to measure the effects of α_1 -adrenoceptor antagonists on cardiovascular endpoints and to characterize the pharmacokinetics of CHIR 2279. To assess cardiovascular changes, MAP and heart rate were monitored in conscious, freely mobile animals by radiotelemetry (Data Sciences International, St. Paul, MN). A few days before the experiments, a sensor was surgically inserted into the abdominal aorta and a transmitter implanted in the peritoneal cavity. The sensor consisted of flexible leads that detected pressure fluctuations in the aorta; the transmitter was used to send digitized data by radiofrequency signals to a receiver beneath the cage. For administration of the test compounds, each animal was configured with a jugular vein cannula (Harms and Ojeda, 1974). MAP and heart rate were measured in each animal over a 10-sec interval every 1 to 2 min for several days. To characterize the base-line condition of the cardiovascular parameters, data were collected 24 hr before dosing. The average values for MAP and heart rate observed during this 24-hr base-line period were used to

normalize measurements obtained after dosing. For rats, base-line values for MAP typically averaged about 100 mm Hg and heart rate was approximately 430 beats per min. For guinea pigs, base-line MAP was about 65 mm Hg and heart rate was about 310 beats per min. After the data from individual animals were normalized, they were averaged with data from other animals in the same treatment to obtain an estimate of the average cardiovascular response to a treatment at each sample time after dosing.

Approximately 24 hr before the pharmacokinetics experiments, rats and guinea pigs were configured with cannulae (Bauer *et al.*, 1994). A jugular vein cannula was implanted in the rats for blood sampling, and the guinea pigs received a jugular and femoral vein cannula for blood sampling and dosing, respectively. The cannulae were flushed and filled with sterilized saline containing 10 U/ml heparin (sodium injection, USP heparin, Elkins-Sinn, Inc., Cherry Hill, NJ) both after the implantation and between the blood samplings. For blood sampling, a 0.25-ml volume was pulled through the cannula immediately before sampling to clear the line and a 0.2-ml sample of blood was removed with a fresh syringe. Blood samples were transferred to 0.5-ml Eppendorf tubes containing 15 μl heparin (1 U/ml) and then centrifuged for 2 min in a table top microfuge at 4°C. The plasma was removed, transferred to a fresh tube and stored at -70°C.

In both the cardiovascular and pharmacokinetic experiments, the vehicle used to deliver CHIR 2279 was a mixture of 10% ethanol (200 proof, Gold Shield Chemical Co., Hayward, CA), 40% polyethylene glycol-400 (Aldrich Chemical Co., Milwaukee, WI) and 50% acetate-buffered saline (0.9% sodium chloride, 0.012% glacial acetic acid, pH adjusted to 5.2 ± 0.2). The *in vivo* properties of this vehicle have been described previously (Fu *et al.*, 1987). In the cardiovascular experiments, the doses were administered as a rapid i.v. bolus into a jugular vein cannula for both species. In the pharmacokinetics experiments, rats were administered the dose through a prominent lateral tail vein, and guinea pigs were dosed through a femoral vein cannula. In both cardiovascular and pharmacokinetic experiments, the dosing volume was 2 ml/kg in the rats and 1 ml/kg in the guinea pigs.

In the pharmacokinetic experiments, the plasma concentrations of CHIR 2279 were measured by reverse-phase HPLC. In the initial procedure, 0.1 ml of plasma was amended with 1 μg internal standard and diluted to 0.55 ml with 5.88% acetonitrile in Tris buffer (pH 7.2). A 0.5-ml volume of this mixture was passed onto a HiSep C18 column (Supelco, Fullerton, CA) and washed for 10 min at 0.1 ml/min with 5% acetonitrile in 0.5 M Tris (pH 7.2). The HiSep column was then connected to a reverse-phase HPLC column (Reliasil, Michrom BioResources, Inc., Auburn, CA) attached to a microbore HPLC (Michrom BioResources, Inc.). The two buffers for chromatography on the Reliasil column were: 1) HPLC buffer A, 2% acetonitrile in water, 0.1% diisopropyl ethylamine, 0.1% formic acid; and 2) HPLC buffer B, 98% acetonitrile in water, 0.09% diisopropyl ethylamine, 0.09% formic acid. A gradient was implemented with a gradual change from 35 to 55% HPLC buffer B over 20 min, and elution of CHIR 2279 was monitored at 254 nm. A calibration curve that related plasma concentrations of CHIR 2279 to HPLC peak areas showed linearity to 30 ng/ml, and the precision of the assay was $\pm 9\%$.

The PK/PD calculations were performed with ADAPT II mathematical software (Biomedical Simulations Resource, University of Southern California, Los Angeles, CA). Previously described methods (D'Argenio and Schumitzky, 1979) were used to estimate system and variance parameters via maximum likelihood, and the S.E. of the estimates were approximated via generalized least squares.

For both rats and guinea pigs, PK/PD calculations were performed by simultaneously fitting the pharmacokinetic and pharmacodynamic data obtained from all animals administered i.v. CHIR 2279 at 10 mg/kg. The pharmacokinetic data were described by a two-compartment model with rapid bolus input and first-order output

² This observation is consistent with results published by Miller *et al.*, 1994, demonstrating stability of NSG-peptoids against cleavage by gastrointestinal peptidases.

from the central compartment. The two differential equations describing net mass transfer of compound for this model were:

$$\frac{dX_1}{dt} = -K_{12} \cdot X_1 + K_{21} \cdot X_2 - K_{10} \cdot X_1$$

$$\frac{dX_2}{dt} = -K_{21} \cdot X_2 + K_{12} \cdot X_1$$

where X_1 was the amount (ng/kg) of compound in the central compartment; X_2 was the amount (ng/kg) of compound in the peripheral compartment; t was the time after the injection; K_{12} was the first-order distribution rate constant for transfer from the central to the peripheral compartment (hr^{-1}); K_{21} was the first-order distribution rate constant for transfer from the peripheral to the central compartment (hr^{-1}) and K_{10} was the first-order rate constant for elimination from the central compartment (hr^{-1}). The plasma concentration (C_1) of compound was related to X_1 by:

$$C_1 = \frac{X_1}{V_c}$$

where V_c was the volume of the central compartment in units of ml/kg. CL , V_β , $T_{1/2\alpha}$ and $T_{1/2\beta}$ were derived from the estimated pharmacokinetic parameters as described by Rowland and Tozer (1989).

Two basic models were examined for characterizing the pharmacodynamic response observed after administration of CHIR 2279: an indirect response model and a link model. The indirect response model (Dayneka *et al.*, 1993) assumed that the reduction in MAP induced by CHIR 2279 was caused by an inhibition of endogenous mechanisms that ordinarily maintain MAP at base-line values. This model was rejected on the basis of goodness of fit (*i.e.*, the predicted values were poorly correlated with the observed values; $R < 0.4$).

Using an approach described by Holford and Sheiner (1981), pharmacokinetic and pharmacodynamic data were simultaneously fit using a link model. The link model postulates a hypothetical effect compartment linked to the plasma compartment by a first-order process, but whose exponential does not enter into the pharmacokinetic solution for the mass of compound in the body. The amount of compound in the effect compartment was described by the differential equation:

$$\frac{dX_3}{dt} = -K_{1e} \cdot X_1 - K_{e0} \cdot X_3$$

where K_{1e} was the first-order rate constant for the hypothetical transfer of drug from the central to the effect compartment, K_{e0} was the first-order rate constant for dissipation of the compound from the effect compartment, and X_3 was the amount (ng/kg) of compound in the effect compartment. The concentration of compound in the effect compartment (C_e) was related to X_3 by

$$C_e = \frac{X_3}{V_e/K_{e0}}$$

The pharmacodynamic half-life ($T_{1/2K_{e0}}$) was derived by dividing $\ln 2$ by K_{e0} . The hypothetical amount of drug in the effect compartment was related to the observed effect by the Hill equation:

$$E = \frac{E_{\max} \cdot C_e^\gamma}{EC_{50} + C_e^\gamma}$$

where E was the magnitude of the pharmacologic effect (percent change in MAP), E_{\max} was the maximum pharmacologic effect observed, C_e was the concentration (ng/ml) of compound in the effect compartment, γ was the sigmoidicity parameter, and EC_{50} was the concentration in the effect compartment at which 50% of the maximum pharmacologic effect was observed. During the curve-fitting

E_{\max} was fixed at the experimentally observed maximum value for blood pressure depression (22.8% for rats and 54.0% for guinea pigs).

Pressor response experiments. To establish whether the hypotensive effects of CHIR 2279 were correlated with α_1 -adrenoceptor blockade, pressor response experiments were performed with phenylephrine. Because the maximal pharmacological responses induced by CHIR 2279 and phenylephrine were known to be transient, interaction studies were performed with continuous i.v. infusions of CHIR 2279 and intermittent i.v. bolus doses of phenylephrine. MAP was measured by radiotelemetry as previously described. Continuous i.v. infusion in conscious rats generally requires the use to restraints, a condition that could not be met given the abdominal placement of the transmitters for radiotelemetry. The rats were therefore subject to complete anesthesia with 1% isoflurane gas during the pressor experiments. Continuous infusions were performed at a rate of 0.06 ml/min/kg with either standard vehicle alone (10% ethanol, 40% PEG 400 and 50% acetate-buffered saline) or standard vehicle amended with CHIR 2279. For the infusions of CHIR 2279, the concentration of CHIR 2279 in the vehicle was adjusted for each rat to a level that would result in an infusion rate of 0.3 mg/kg/min. With continuous i.v. infusions, plasma concentrations reach 90% of the steady-state concentration after four elimination half-lives. Inasmuch as characterization of the pharmacokinetics of CHIR 2279 showed the effective elimination half-life in rats to be 30 min, infusions of CHIR 2279 were performed for at least 120 min before administration of the phenylephrine. Bolus doses of i.v. phenylephrine were administered in standard vehicle in a volume of 0.2 ml/kg at 1, 3, 10, 30, 100 and 300 $\mu\text{g}/\text{kg}$, and MAP was measured in 20-sec intervals by radiotelemetry. Maximum increases in MAP induced by phenylephrine were generally observed within 60 sec of administration of the agonist and resolved to base line within 2.5 min. The percentage change in MAP induced by phenylephrine was calculated as the fractional increase in MAP observed at the peak response relative to the average MAP observed for the 2 min preceding the bolus dosing.

Results

Radioligand binding assays. Binding characteristics of the LTK-derived membranes were compared with homogenates of rat submaxillary gland. Figure 2 presents a typical binding profile of [^3H]prazosin to the cloned receptors and shows that binding of [^3H]prazosin to LTK⁻ membranes was saturable and of high affinity. As shown in table 1, K_d values were similar for rat submaxillary gland and LTK⁻ cells transfected with either α_{1a} , α_{1b} , or α_{1d} receptors (averaging about 60 pM), but the receptor density was much higher for the LTK⁻ membranes.

To characterize the binding affinity of CHIR 2279 to various α_1 -adrenoceptor subtypes, competitive binding assays were performed with [^3H]prazosin (fig. 3). As summarized in table 2, CHIR 2279 was similar to prazosin in showing high affinity for each of the receptor subtypes tested. However, comparison of the K_i values shows CHIR 2279 to have approximately 10-fold lower affinity for α_1 -adrenoceptor subtypes than prazosin. WB-4101 and 5-methyl urapidil each showed greater specificity of binding than CHIR 2279, with high affinity for α_{1a} and α_{1A} , intermediate affinity for α_{1d} , and weakest affinity for α_{1b} . Norepinephrine, the endogenous ligand, showed selectivity for α_{1d} , but K_i values that were typically 100-times weaker than those observed with CHIR 2279.

Competitive binding assays at three subtypes of α_2 -adrenoceptors (table 3) showed that CHIR 2279 had considerably lower affinity for α_2 - than for α_1 -receptors (table 2). In addi-

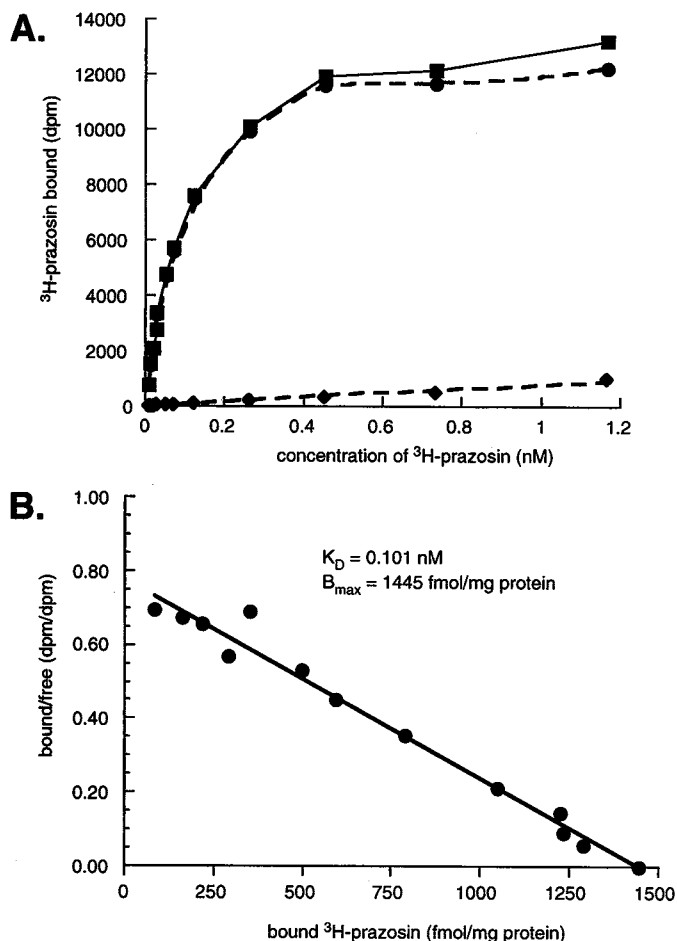


Fig. 2. Radioligand binding of [^3H]prazosin to LTK $^-$ cells transfected with the bovine α_{1a} adrenoceptor. A, representation of total binding (■), nonspecific binding (◆) and specific binding (●). B, Scatchard plot of the data shown in A.

TABLE 1
Radioligand binding affinities for [^3H]prazosin and receptor densities in rat submaxillary gland (α_{1A}) and membranes derived from transformed LTK cells (α_{1a} , α_{1b} , α_{1d})

Receptor Subtype	N	K_d	B_{max}	B_{max}
		pM	fmol/mg ww	fmol/mg protein
α_{1A}	6	59.4 (40.1–88.0)	3.25 ± 0.98	42.6 ± 6.19
α_{1a}	7	117.0 (96.8–140)	92.7 ± 10.2	2260 ± 560
α_{1b}	4	50.8 (40.3–63.9)	45.0 ± 4.86	429 ± 38.9
α_{1d}	4	67.3 (58.3–77.8)	26.9 ± 6.7	296 ± 47.3

Average values are reported \pm s.e.m. Numbers in parentheses indicate 95% confidence intervals.

tion, CHIR 2279 was more than two orders of magnitude less potent at α_2 -adrenoceptors than standard antagonists such as rauwolfscine or phentolamine and was several-fold less potent than the reference agonist, NE (table 3).

Competitive binding assays with both β_1 - and β_2 -adrenoceptors (table 4) showed that CHIR 2279 had measurable binding to both receptors, but the K_i values indicated very low affinities (7000 nM at each receptor). CHIR 2279 was more than two orders of magnitude less potent at both β_1 and β_2 receptors than either the agonist isoproterenol or the antagonist propranolol.

Competitive binding assays using 5HT $_1$ and 5HT $_2$ recep-

tors (table 5) showed that CHIR 2279 had very low affinity ($>10,000$ nM) at each receptor. In comparison, serotonin had high affinity for 5HT $_1$ sites, although ketanserin demonstrated high affinity for 5HT $_2$ sites, and the relatively non-selective antagonist, methysergide, had high affinity for each subtype of serotonin receptor (table 5).

Table 6 summarizes the results of assays for inhibition of competitive biogenic amine uptake for NE, 5HT and DA into synaptosomes. These assays showed that CHIR 2279 lacked potent activity as an inhibitor of biogenic amine uptake. In contrast, desipramine potently inhibited NE uptake, fluoxetine selectively blocked 5HT uptake and the potent NE/DA uptake inhibitor, mazindol, showed moderate potency at 5HT uptake sites as well. Table 6 also lists the relative potencies of the endogenous biogenic amines for their respective uptake sites.

Isolated tissue bioassays. CHIR 2279 was evaluated for its antagonistic activity at postsynaptic α_{1A} - or α_{1B} -adrenoceptors in isolated rat vas deferens, canine prostate strips (both α_{1A}), rat spleen strips (α_{1B}) and rat aortic rings (α_{1D}). CHIR 2279 was approximately equipotent in antagonizing agonist-induced contractile responses in these four tissues (table 7) and was about 10- to 100-fold less potent than prazosin. As shown in figures 4 to 7, the antagonism of agonist-induced responses by CHIR 2279 appeared competitive in rat vas deferens, canine prostate, rat spleen and rat aorta, respectively.

IUP in dogs. CHIR 2279 antagonized the increase in IUP induced by epinephrine in anesthetized dogs (fig. 8). Doses of 0.3 to 3.0 mg/kg i.v. resulted in dose-dependent dextral shifts of the agonist dose-response curves, which were consistent with antagonism of postsynaptic α_1 adrenoceptors in the prostatic and/or urethral smooth muscle. The shifts in the curves for epinephrine response were approximately 4.0-, 11.1- and 47-fold, respectively, for 0.3, 1.0 and 3.0 mg/kg doses of CHIR 2279, where the dose of epinephrine was sufficient to raise IUP by 50% (approximately 25–30 mm Hg). These shifts in the agonist-induced IUP response could be used to calculate an *in vivo* pseudo pA_2 value of 6.86. By comparison, prazosin (data not shown) produced an *in vivo* pseudo pA_2 of 7.71, a finding that is consistent with its greater potency observed *in vitro* (table 7).

MAP and heart rate in rats and guinea pigs. The cardiovascular effects of vehicle alone, CHIR 2279 and prazosin were evaluated in rats and guinea pigs. When compared directly to the effects of vehicle alone, administration of CHIR 2279 caused significant depressions in MAP (fig. 9) and heart rate (fig. 10) in both species. Compared to the vehicle, prazosin also caused a significant reduction in MAP in rats and guinea pigs (fig. 9), but although heart rate decreased in guinea pigs dosed with prazosin, in rats, prazosin caused an increase in heart rate (fig. 10).

Dose-response relationships for MAP with i.v. bolus administration of both CHIR 2279 and prazosin are summarized in figure 11. In rats, the range of effective doses of CHIR 2279 (1–10 mg/kg) was approximately 1000-times higher than that of prazosin (1–10 $\mu\text{g}/\text{kg}$). At doses higher than those shown in figure 11, greater depressions were not observed in MAP, suggesting that the maximum depression in MAP in normotensive rats was 23%. Similarly, the maximum depression of MAP in guinea pigs was 54%. Guinea pigs generally appeared to be more sensitive than rats to MAP

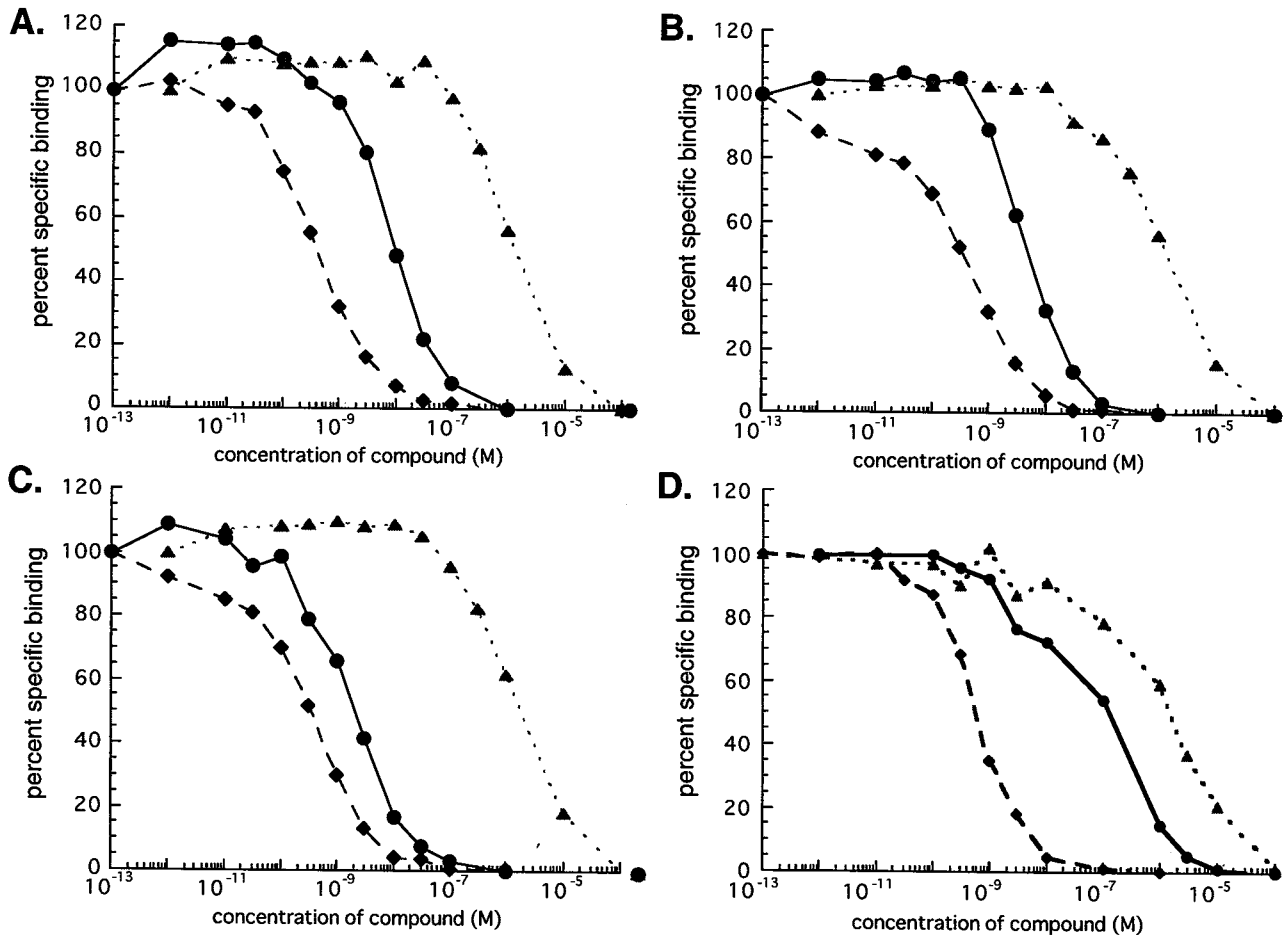


Fig. 3. Competition binding between [3 H]prazosin, CHIR 2279 (●), prazosin (◆) or norepinephrine (▲) with various subtypes of α_1 -adrenoceptors. The α_1 -adrenoceptor subtypes were: A, α_{1A} receptors from rat submaxillary gland; B, cloned hamster α_{1b} ; C, cloned bovine α_{1a} ; or D, cloned rat α_{1d} . For all receptor subtypes, the membranes were incubated in the presence of 0.2 nM [3 H]prazosin and various concentrations of each competitive agent as described in "Materials and Methods."

TABLE 2

Receptor binding affinities of CHIR 2279 and representative standard compounds for subtypes of α_1 -adrenoceptors

Compound	Receptor Subtype (nM)			
	α_{1A}	α_{1a}	α_{1b}	α_{1d}
CHIR 2279	2.42 (1.96–2.97; 7)	0.705 (0.565–0.880; 7)	1.28 (0.767–2.13; 8)	2.88 (1.51–5.49; 4)
Prazosin	0.125 (0.052–0.302; 7)	0.194 (0.135–0.279; 16)	0.054 (0.036–0.081; 16)	0.100 (0.065–0.153; 12)
5-Methyl urapidil	1.45 (0.642–3.28; 5)	0.613 (0.394–0.953; 7)	41.8 (31.4–55.6; 7)	14.3 (9.19–22.3; 7)
WB-4101	0.098 (0.026–0.371; 6)	0.044 (0.021–0.09; 8)	0.630 (0.311–1.28; 6)	0.120 (0.059–0.241; 6)
Norepinephrine	253 (232–276; 138)	450 (402–504; 145)	284 (261–311; 143)	21.0 (17.9–24.6; 139)

Mean K_i values are reported. Numbers in parentheses indicate 95% confidence intervals and the number of replications.

TABLE 3

Receptor binding affinities of CHIR 2279 and representative standard compounds for subtypes of α_2 -adrenoceptors

Compound	Receptor Subtype (nM)		
	α_{2a}	α_{2B}	α_{2c}
CHIR 2279	168 (59.8–474; 4)	1440 (733–2820; 4)	150 (37.2–603; 4)
Rauwolscine	2.369 (1.06–5.27; 6)	0.566 (0.338–0.947; 7)	0.585 (0.419–0.816; 6)
Phentolamine	0.590 (0.315–1.10; 5)	0.867 (0.539–1.40; 7)	2.38 (1.52–3.72; 5)
Norepinephrine	56.3 (51.1–62.1; 103)	22.4 (20.3–24.7; 190)	10.2 (8.68–12.0; 105)

Mean K_i values are reported. Numbers in parentheses indicate 95% confidence intervals and the number of replications.

reduction by CHIR 2279; at 0.1 mg/kg in guinea pigs CHIR 2279 caused MAP to drop 10 to 20% for about 30 min, but in rats 1 mg/kg had no effect on MAP.

To establish whether the hypotensive effects of CHIR 2279

were correlated with α_1 -adrenoceptor blockade, pressor response experiments were performed in rats with phenylephrine, an α_1 -specific agonist (Bethelsen and Pettinger, 1977; vanZwieten, 1988). As shown in figure 12, higher doses of

TABLE 4
Receptor binding affinities of CHIR 2279 and representative standard compounds for subtypes of β -adrenoceptors

Compound	Receptor Subtype (nM)	
	β_1	β_2
CHIR 2279	7290 (1780–29,800; 3)	6530 (4380–9750; 3)
Propranolol	4.29 (1.71–10.6; 6)	0.879 (0.289–2.67; 3)
Isoproterenol	31.0 (22.4–42.9; 9)	125 (79.6–196; 9)
Norepinephrine	369 (317–430; 79)	2650 (2310–3040; 76)

Mean K_i values are reported. Numbers in parentheses indicate 95% confidence intervals and the number of replications.

TABLE 5
Receptor binding affinities of CHIR 2279 and representative standard compounds for subtypes of 5HT-receptors

Compound	Receptor Subtype	
	5HT ₁	5HT ₂
CHIR 2279	>30,000 (3)	10,920 (6850–17,400; 3)
Serotonin	3.59 (3.24–3.97; 134)	2730 (2380–3130; 52)
Ketanserin	>10000 (1)	0.695 (0.552–0.875; 4)
Methysergide	24.0 (9.99–57.6; 4)	2.06 (1.20–3.54; 6)

Mean K_i values are reported. Numbers in parentheses indicate 95% confidence intervals and the number of replications.

phenylephrine were needed to cause increases in MAP when phenylephrine was coadministered with CHIR 2279.

In both rats and guinea pigs, the plasma pharmacokinetics of CHIR 2279 were adequately described by a two-compartment mammillary³ model (fig. 13A and table 8). The $T_{1/2\alpha}$ (1 min) and $T_{1/2\beta}$ (50 min) were similar in the two species, but the systemic clearance rate was approximately twice as fast in guinea pigs than rats (104 versus 60 ml/min · kg).

As shown in figure 13B, immediately after administration of CHIR 2279 by i.v. bolus, a 4- and 10-min delay occurred in the maximum depression in MAP in guinea pigs and rats, respectively. The delay between the maximum plasma concentration of CHIR 2279 and the maximum effect was due to a temporal lag in the equilibration of CHIR 2279 from the plasma to the effect compartment. The magnitude of this delay corresponded to the difference between the pharmacokinetic half-lives ($T_{1/2\alpha}$ and $T_{1/2\beta}$) and the pharmacodynamic half-life ($T_{1/2K_{e0}}$) of CHIR 2279. In guinea pigs, $T_{1/2K_{e0}}$ was smaller than both $T_{1/2\alpha}$ and $T_{1/2\beta}$, (table 8) which was consistent with the observed rapid onset of the maximum effect after i.v. bolus dosing. In rats, $T_{1/2K_{e0}}$ was smaller than $T_{1/2\alpha}$, but larger than $T_{1/2\beta}$ (table 8). This finding in rats was consistent with the observed delay in the maximum effect after i.v. dosing and indicated that the onset of the effect was slower than the systemic distribution of CHIR 2279.

Figure 13 shows the results of simultaneous curve fits of the pharmacokinetic and pharmacodynamic data. That the PK/PD model described the data well is evidenced by the large R-squared values and the small coefficients of variation corresponding to the estimated parameters (table 8). As shown in figure 13C, the curve describing the relationship between concentration and effect was shifted to the left in guinea pigs relative to rats, and the corresponding EC_{50} was 50% lower in guinea pigs (520 vs. 1170 ng/ml; table 8). Based

³ In classical pharmacokinetic models a mammillary system is described as one in which all of the blood (or plasma) is assumed to be located in a central compartment (Roth *et al.*, 1995).

on this result, CHIR 2279 appears to be twice as potent in guinea pigs than rats at reducing MAP.

Discussion

The endogenous ligands for the α_1 -adrenoceptors are epinephrine and norepinephrine (fig. 1B). As observed previously by Zuckermann and coworkers (1994), epinephrine and CHIR 2279 share a substituted phenylethylamine. However, the binding of CHIR 2279 appears not to be due to the tyramine residue alone, because CHIR 2279 had only slightly weaker binding affinities in a competitive receptor-binding assay with [³H]prazosin in rat forebrain. Furthermore, tyramine itself did not compete for binding at a concentration of 10 μ M (Zuckermann *et al.*, 1994). Receptor-binding assays in our report (table 2 and fig. 3) show that CHIR 2279 potently competes with prazosin, a small molecule heterocycle that has no apparent structural homology to CHIR 2279 (fig. 1C). As with prazosin, CHIR 2279 showed nanomolar-affinity for α_{1A} -, α_{1A} -, α_{1B} - and α_{1D} -adrenoceptor subtypes, but the K_i values were 10-fold weaker with CHIR 2279.

In contrast to the α_1 -adrenoceptor assays, competitive binding assays with CHIR 2279 showed negligible binding affinities (K_i values > 6500 nM) for β_1 - and β_2 -adrenoceptors (table 4) as well as 5HT₁ and 5HT₂ receptors (table 5). Similarly, binding assays with α_{2A} , α_{2B} and α_{2C} (table 3) showed that CHIR 2279 was at least 50-fold more potent at binding α_1 - than α_2 -receptors. In addition to the competition binding assays cited in this report, combinatorial mixtures containing CHIR 2279 have been tested with a broad range of 7-transmembrane G-protein coupled receptors. The results of these combinatorial assays indicate that CHIR 2279 generally lacks high affinity binding ($K_i > 1 \mu$ M) for nonadrenergic receptors in this class. Overall, the results of competition binding assays suggest that CHIR 2279 has specificity of binding toward α_1 -adrenoceptors.

Certain clinically effective antidepressants are thought to produce their therapeutic effects by increasing the concentrations of NE and 5HT in the synaptic cleft (Richelson, 1987; Pinder and Wieringa, 1993). Antagonists of α_2 -adrenoceptors have been proposed as potentially useful antidepressants since they can enhance synaptic NE and/or 5HT levels by blocking feedback inhibition processes (Wiech and Ursillo, 1980). Because CHIR 2279 was shown to have weak affinity of binding for α_2 -receptors (table 3), NE reuptake properties were examined as part of its pharmacologic characterization. As shown in table 6, however, CHIR 2279 lacked potent activity as an inhibitor of the uptake of biogenic amines.

Isolated rat vas deferens, rat spleen, rat aorta and canine prostate were used to characterize the functional activity of CHIR 2279 (table 7). Two of these bioassays, rat vas deferens and rat spleen, are considered classical tests for α_{1A} - and α_{1B} -mediated responses, respectively (Bylund *et al.*, 1994). The rat aorta has been suggested to represent a model of α_{1D} -adrenoceptors (Aboud *et al.*, 1993; Buckner *et al.*, 1995), and assays involving canine prostate are aimed at assessing α_{1A} - (Brune *et al.*, 1995) and α_{1L} - (Muramatsu *et al.*, 1995) mediated effects. As observed with the binding assays (table 2), the bioassays showed that CHIR 2279 was generally 10- to 100-fold less potent than prazosin (table 7). Unlike the receptor-binding assays, the tissue bioassays with WB-4101 failed to show discrete preferences for the α_1 -adrenoceptor

TABLE 6

Amine uptake potencies of CHIR 2279 and representative standard compounds for biogenic amine uptake sites

Compound	Uptake Site (nM)		
	NE	5HT	DA
CHIR 2279	8100 (4590–14,300; 4)	>30,000 (2)	4960 (350–69,000; 3)
Desipramine	11.6 (8.66–15.7; 43)	6280 (3970–9930; 5)	90,100 (68,300–120,000; 5)
Fluoxetine	3280 (602–17,800; 5)	308 (255–371; 48)	16,200 (13,800–19,000; 3)
Mazindol	5.21 (0.26–104; 4)	827 (614–1110; 4)	243 (177–333; 4)
Norepinephrine	574 (493–667; 53)	40,600 (9650–170,000; 3)	843 (210–3390; 3)
Serotonin	20900 (7290–60,100; 3)	413 (338–504; 54)	8300 (3840–17,900; 3)
Dopamine	428 (319–576; 3)	>8500 (2)	323 (262–398; 39)

NE, Norepinephrine; 5HT, serotonin and DA, dopamine. Mean IC_{50} values are reported. Numbers in parentheses indicate 95% confidence intervals and the number of replications.

TABLE 7

Antagonist potencies of CHIR 2279 and representative standard compounds for subtypes of α_1 -adrenoceptors in selected isolated tissues *in vitro*

Compound/Parameter	Receptor Subtype			
	α_{1A} Rat vas deferens	α_{1A} Canine prostate	α_{1B} Rat spleen	α_{1D} Rat aorta
CHIR 2279				
pA ₂	6.55 ± 0.40	7.32 ± 0.17	7.35 ± 0.50	6.81 ± 0.24
Slope	0.90 ± 0.24	0.66 ± 0.07	0.63 ± 0.13	1.20 ± 0.16
N	22	24	26	11
Prazosin				
pA ₂	8.78 ± 0.30	8.48 ± 0.24	10.02 ± 0.16	9.35 ± 0.20
Slope	1.17 ± 0.15	0.95 ± 0.08	1.07 ± 0.15	1.18 ± 0.09
N	15	14	13	10
5-Methyl urapidil				
pA ₂	8.39 ± 0.14	8.76 ± 0.30	7.17 ± 0.10	7.83 ± 0.12
Slope	1.00 ± 0.09	0.94 ± 0.12	1.01 ± 0.06	1.04 ± 0.07
N	14	10	27	9
WB-4101				
pA ₂	9.69 ± 0.40	8.69 ± 0.25	9.39 ± 0.26	9.36 ± 0.30
Slope	0.86 ± 0.12	1.3 ± 0.13	0.91 ± 0.08	1.15 ± 0.12
N	13	10	20	8

Average pA₂ values are reported ± S.E.M.

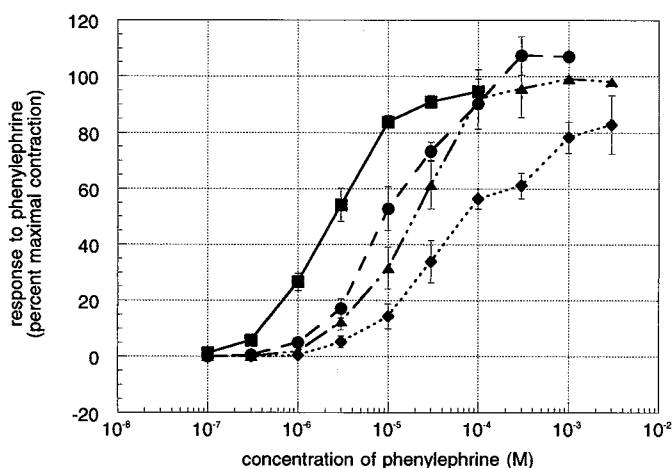


Fig. 4. Effect of CHIR 2279 on phenylephrine-induced contractions in isolated rat vas deferens. Cumulative concentrations of phenylephrine were added to tissue baths containing rat vas deferens as described in "Materials and Methods." Concentrations of CHIR 2279 were: 0 μ M (■; $n = 8$); 0.3 μ M (●; $n = 6$); 1 μ M (▲; $n = 8$); and 3 μ M (◆; $n = 8$). After a 30-min equilibration, tissues were exposed to a repeated agonist challenge. Values shown are the means ± S.E.M.

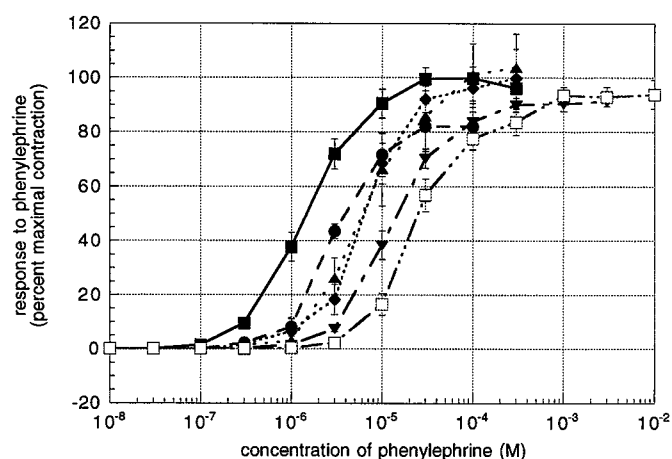


Fig. 5. Effect of CHIR 2279 on phenylephrine-induced contractions in isolated canine prostate strips. Cumulative concentrations of phenylephrine were added to tissue baths containing strips of canine prostate as described in "Materials and Methods." Concentrations of CHIR 2279 were: 0 μ M (■; $n = 6$); 0.03 μ M (●; $n = 2$); 0.1 μ M (▲; $n = 6$); 0.3 μ M (◆; $n = 4$); 1 μ M (▼; $n = 6$) and 3 μ M (□; $n = 6$). After a 30-min equilibration, tissues were exposed to a repeated agonist challenge. Values shown are the means ± S.E.M.

subtypes. This finding is likely attributable to the lack of homogeneity of α_1 -adrenoceptor subtypes in the tissues (Mineman, 1988; Wilson *et al.*, 1991).

When functional α_1 -adrenoceptor antagonist affinity of

CHIR 2279 is compared to its affinity for native or recombinant α_1 -adrenoceptors (as determined by competitive binding assays), the functional potency is 35- to greater than 300-fold lower (compare tables 2 and 7). Because CHIR 2279 was

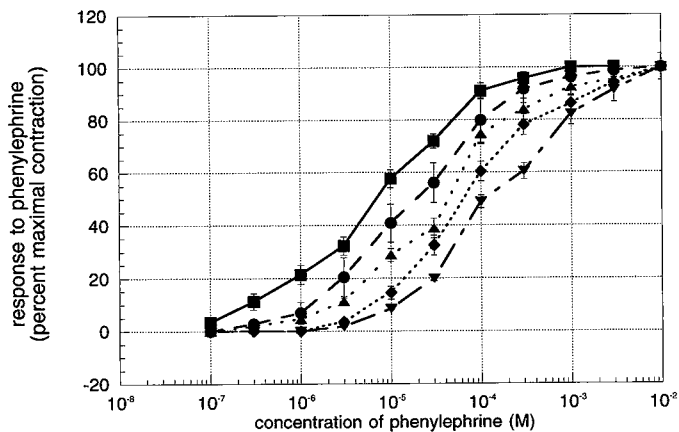


Fig. 6. Effect of CHIR 2279 on phenylephrine-induced contractions in isolated rat splenic strips. Cumulative concentrations of phenylephrine were added to tissue baths containing strips of rat spleen as described in "Materials and Methods." Concentrations of CHIR 2279 were: 0 μM (■; $n = 8$); 0.1 μM (●; $n = 4$); 0.3 μM (▲; $n = 6$); 1 μM (◆; $n = 8$) and 3 μM (▼; $n = 8$). After a 30-min equilibration, tissues were exposed to a repeated agonist challenge. Values shown are the means \pm S.E.M.

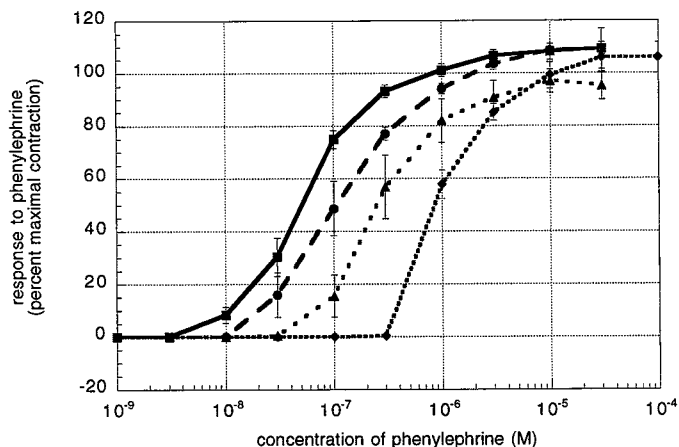


Fig. 7. Effect of CHIR 2279 on phenylephrine-induced contractions in isolated rat aortic rings. Cumulative concentrations of phenylephrine were added to tissue baths containing rings of rat aorta as described in "Materials and Methods." Concentrations of CHIR 2279 were: 0 μM (■; $n = 4$); 0.3 μM (●; $n = 4$); 1 μM (▲; $n = 4$) and 3 μM (◆; $n = 3$). After a 30-min equilibration, tissues were exposed to a repeated agonist challenge. Values shown are the means \pm S.E.M.

shown to be both soluble and metabolically stable in the *in vitro* assays (see "Materials and Methods") the relatively weaker potency in the functional assays is most likely related to access to the receptor in intact tissue. The lack of correlation between functional and binding assays suggests that access of CHIR 2279 to the receptor in native tissues may be less than observed with other α_1 -adrenoceptor antagonists. However, it should be noted that this low degree of correlation between functional and binding assays is consistent with other α_1 -antagonists tested in our laboratory.

Inasmuch as one of the principal treatments for clinical benign prostatic hyperplasia is α_1 -blockade (Lowe and Stark, 1993), it was of interest to characterize the *in vivo* potency of CHIR 2279 at IUP reduction. This was accomplished with a canine model in which the contractile responses to epinephrine were antagonized with CHIR 2279. We (Brune *et al.*, 1995) and others (Breslin *et al.*, 1993) have shown that this model is useful for confirming the pharmacological activity of

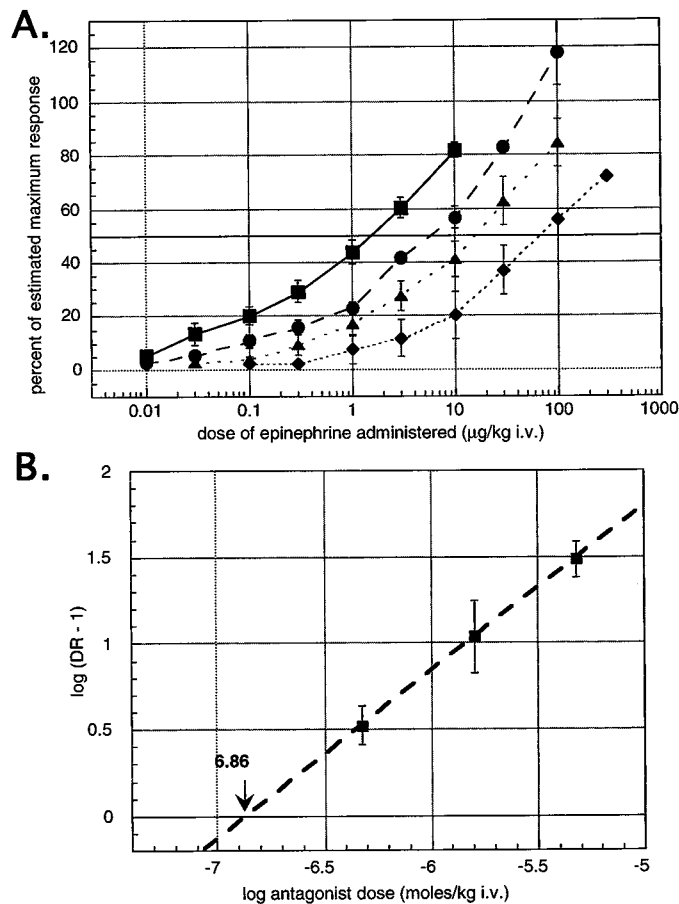


Fig. 8. Effects of CHIR 2279 on IUP in anesthetized dogs. CHIR 2279 was administered cumulatively i.v. to anesthetized dogs and the pressure response within the prostatic urethra to exogenously administered epinephrine was measured as described in "Materials and Methods." A, Increasing doses of CHIR 2279: 0 mg/kg (■; $n = 3$); 0.3 mg/kg (●; $n = 3$); 1 mg/kg (▲; $n = 3$) and 3 mg/kg (◆; $n = 3$). B, Transformation of data shown in panel A to the form of a Schild plot with doses expressed in mol/kg i.v. The calculated pseudo pA_2 value of 6.86 was determined by linear regression analysis.

α_1 -adrenoceptor ligands. As indicated in figure 8, shifts in the agonist-induced IUP response were used to calculate a pseudo pA_2 of 6.86, a value that was 10-fold lower than the pseudo pA_2 associated with prazosin (7.71, data not shown). As indicated in table 7, tissue bioassays with canine prostate indicated a pA_2 of 7.32 with CHIR 2279 and 8.48 with prazosin. Based on these results, it can be concluded that a good correlation exists between the relative prostatic potencies of these two antagonists *in vitro* and *in vivo*.

In addition to use in the treatment of prostatic hyperplasia, a prevalent use of α_1 -adrenoceptor blockade is found in the clinical management of primary hypertension. Unlike the effects α_1 -adrenoceptors on IUP reduction, however, MAP reduction has not been attributed to discrete receptor subtypes and it is possible that vascular contraction involves the contribution of multiple α_1 -adrenoceptor subtypes. Han *et al.* (1990) have assigned receptors of blood vessels to α_{1A} or α_{1B} subtypes. Based on a number of reports, however, it appears that vascular α_1 -adrenoceptors have characteristics that are different from either subtype (Sulpizio and Hieble, 1991; Oriowo and Ruffolo, 1992; Muramatsu *et al.*, 1990).

To assess whether CHIR 2279 had an effect on reduction of

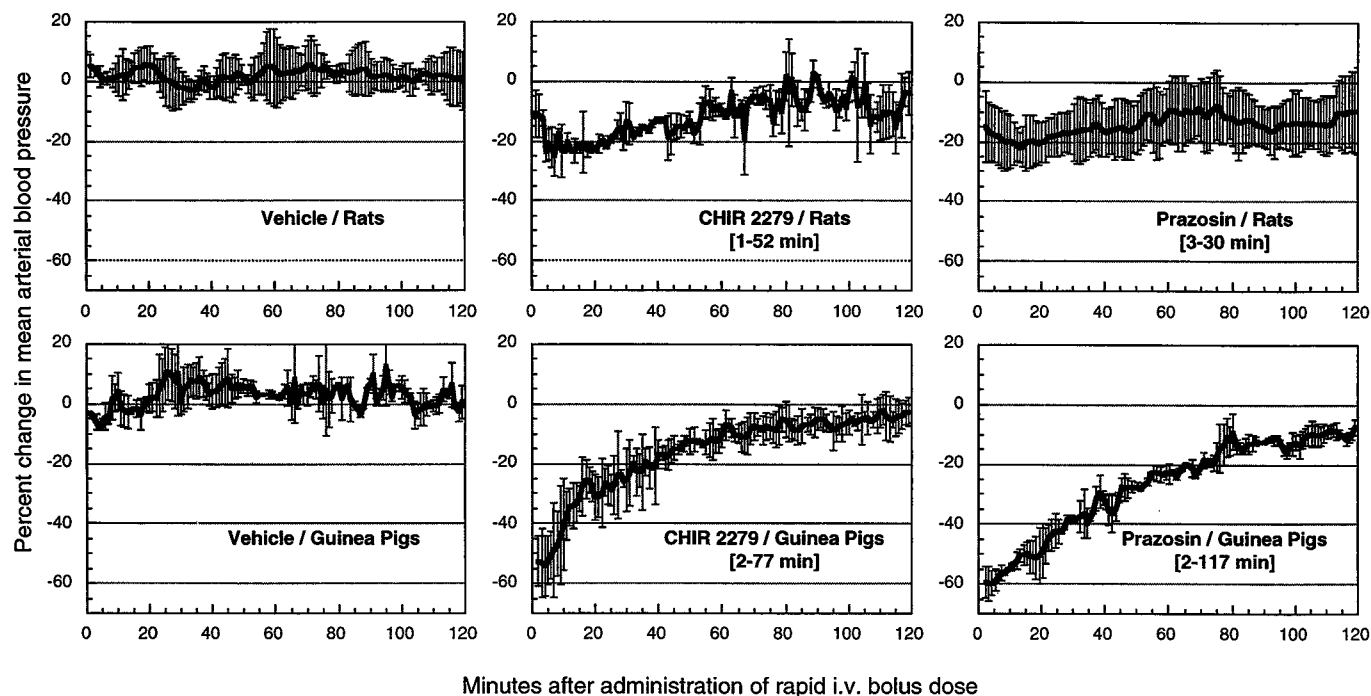


Fig. 9. Changes in mean arterial blood pressure (MAP) after i.v. bolus administration of vehicle, CHIR 2279 and prazosin. MAP changes were assessed by radiotelemetry in normotensive rats and guinea pigs. CHIR 2279 was administered at 10 mg/kg to rats and guinea pigs; prazosin was administered at 0.01 mg/kg in rats and 0.3 mg/kg in guinea pigs. Each point indicates the average of observations made in two to four animals \pm S.D. Numbers in brackets indicate the sample times at which the MAP in the treatment (CHIR 2279 or prazosin) was significantly different from the MAP in the vehicle control treatment ($P < .05$; Student's *t* test).

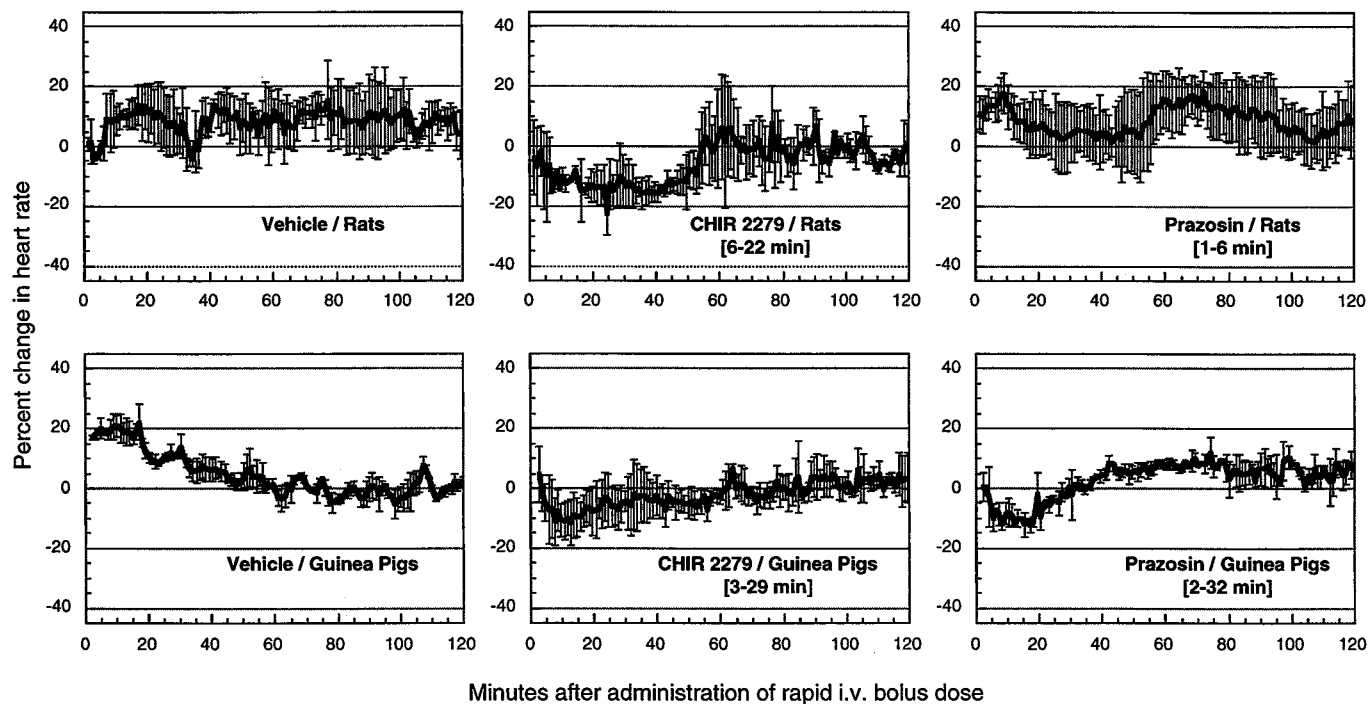


Fig. 10. Changes in heart rate after i.v. bolus administration of vehicle, CHIR 2279 and prazosin. Heart rate changes were assessed by radiotelemetry in normotensive rats and guinea pigs. CHIR 2279 was administered at 10 mg/kg to rats and guinea pigs; prazosin was administered at 0.01 mg/kg in rats and 0.3 mg/kg in guinea pigs. Each point indicates the average of observations made in two to four animals \pm S.D. Numbers in brackets indicate the sample times at which the heart rate in the treatment (CHIR 2279 or prazosin) was significantly different from the heart rate in the vehicle control treatment ($P < .05$; Student's *t* test).

MAP, we examined freely mobile, conscious rats that were monitored by radiotelemetry. Although spontaneously hypertensive rats are commonly used for assessing the hypotensive

effects of pharmacologic agents, we found the magnitude of the changes in MAP and heart rate were similar in normotensive rats. Our cardiovascular experiments therefore fo-

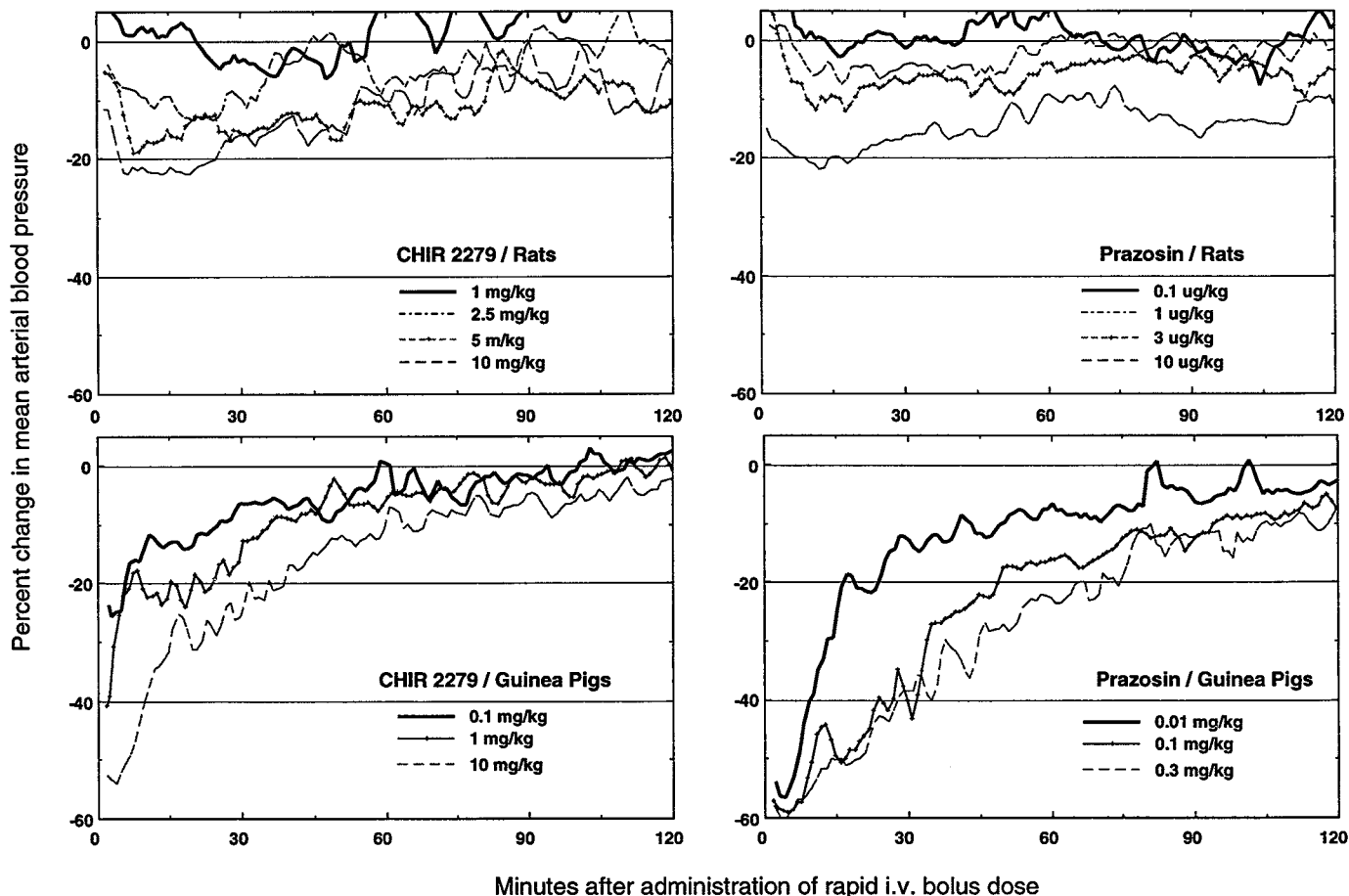


Fig. 11. Dose-response for CHIR 2279 and prazosin for blood pressure reduction. Changes in mean arterial blood pressure (MAP) were assessed by radiotelemetry in normotensive rats and guinea pigs after i.v. bolus administration. Each line indicates the average of observations made in two to four animals.

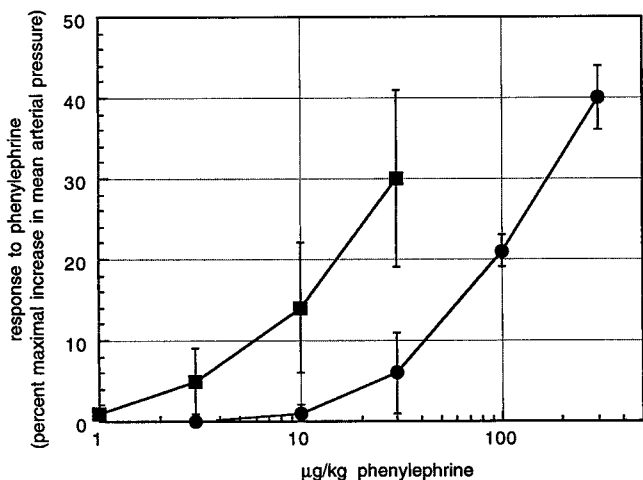


Fig. 12. Effects of CHIR 2279 on phenylephrine-induced hypertension in rats. Phenylephrine was administered as intermittent i.v. bolus doses during a continuous i.v. infusion of either vehicle alone (●) or CHIR 2279 in vehicle (■). As described in "Materials and Methods," the concentration of CHIR 2279 in the vehicle was adjusted for each rat to a level that would result in an infusion rate of 0.3 mg/kg/min. Each point is the average (\pm S.D.) of three measurements in each of three to four rats.

cused on normotensive animals, which proved to be easier than hypertensive strains to surgically configure for the radiotelemetry.

Cardiovascular experiments with rats showed that CHIR 2279 caused reductions in MAP in a dose-dependent manner (fig. 11). In pressor response experiments with phenylephrine, it was shown that the hypertensive effects of this α_1 -selective agonist (Bethelsen and Pettinger, 1977; vanZwieten, 1988) were reduced by coadministration with CHIR 2279 (fig. 12). This antagonism of the pressor response caused by phenylephrine demonstrated that the vasodilatory effects of CHIR 2279 were at least partially driven by α_1 -blockade.

Administration of CHIR 2279 in rats caused virtually synchronized depressions in MAP and heart rate (figs. 9 and 10). This observation contrasted with the results obtained with rats given prazosin, where heart rate increased although MAP dropped. It is interesting to note that although CHIR 2279 and prazosin showed similar binding preferences to various adrenoceptor subtypes (table 2), these two antagonists did not show a similar pattern with respect to induction of bradycardia. Because access to various receptor subtypes *in vivo* is determined by biodistribution, this discrepancy between *in vitro* and *in vivo* observations may be attributed to differences in distribution of the two molecules in various tissues of the animals. In addition, these differences may result from other pharmacological effects that exist between CHIR 2279 and other α_1 antagonists, although our extensive screening has not identified a mechanism that would explain these results.

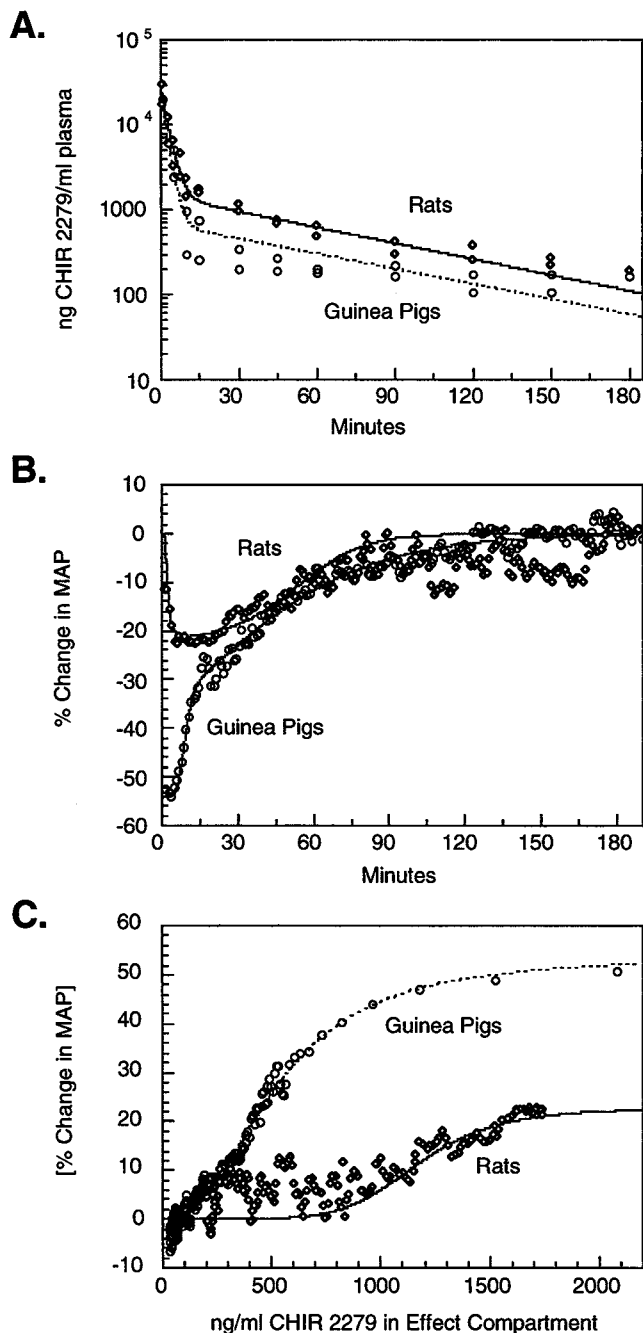


Fig. 13. Pharmacokinetic/pharmacodynamic (PK/PD) analysis of CHIR 2279 in rats and guinea pigs. CHIR 2279 was administered i.v. at 10 mg/kg and changes in mean arterial blood pressure (MAP) were assessed by radiotelemetry. Two animals of each species were used for assessment of the PK, and two to three animals of each species were used to characterize the PD. A and B, PK/PD data and curves obtained from a simultaneous curve-fit of the PK (A) and PD (B) data using a two-compartment model for the PK and an E_{max} -link model for the PD. C, relationships between concentration of CHIR 2279 in the hypothetical effect compartment and absolute changes in MAP.

Terzic *et al.* (1993) have reported that rats express α_1 -adrenoceptors at higher densities in myocardial tissues than other mammalian species; however, data describing the densities of specific subtypes of the receptors are generally lacking. Although rats express α_1 -adrenoceptors at concentrations of about 85 fmol/mg protein, humans, guinea pigs and other mammalian species express these receptors at approx-

TABLE 8

PK/PD parameters obtained with curve-fitting of data from guinea pigs and rats administered CHIR 2279 by i.v. bolus at 10 mg/kg

PK/PD Parameter	Guinea Pigs		Rats	
	Estimate	c.v.	Estimate	c.v.
R-squared, PK	0.954		0.913	
K_{12} (min^{-1})	0.229	2.26%	0.213	0.64%
K_{21} (min^{-1})	0.0287	1.24%	0.0350	1.05%
K_{10} (min^{-1})	0.233	0.54%	0.163	0.70%
V_c (ml/kg)	447	1.82%	369	0.40%
V_β (ml/kg)	7630	a	4170	a
CL (ml/min*kg)	104	a	59.9	a
$T_{1/2\alpha}$ (min)	1.46	a	1.75	a
$T_{1/2\beta}$ (min)	50.8	a	48.3	a
R-squared, PD	0.979		0.743	
K_{eo} (min^{-1})	247	b	0.0275	0.22%
$T_{1/2Keo}$ (min)	0.00281	a	25.2	a
γ	2.38	2.13%	6.37	0.40%
EC_{50} (ng/ml)	520	0.93%	1170	0.05%
E_{max} (% ^{c,d})	54.0	c	22.8	c

Estimated parameters and their corresponding coefficients of variation (c.v.) are reported.

^a Derived from fitted parameters.

^b Too unstable to estimate.

^c Fixed during curve-fitting.

^d % decrease in MAP relative to base line.

imately 10 to 16 fmol/mg protein. Noting that rats express unusually high concentrations of myocardial α_1 -adrenoceptors, it was considered possible that CHIR 2279 primarily affects the rat heart, and that changes in peripheral resistance merely reflect changes in cardiac output. It therefore seemed plausible that the cardiovascular changes observed in rats would not be applicable to other mammalian species, particularly humans.

To assess whether CHIR 2279 elicits hypotensive effects in species that express α_1 -adrenoceptors at concentrations nearer to humans than rats, cardiovascular changes were examined in guinea pigs. In guinea pigs, CHIR 2279 and prazosin each caused reductions in MAP and heart rate (figs. 9 and 10). The data obtained with CHIR 2279 in guinea pigs demonstrate that changes in peripheral resistance induced by CHIR 2279 are not unique to the rat. Combined, the observations from rats and guinea pigs show that CHIR 2279 generally reduces heart rate at doses that are effective at causing depressions in MAP. This finding contrasts CHIR 2279 to other α_1 -blockers, which commonly elicit tachycardia at doses that reduce blood pressure (Bateman *et al.*, 1979; Seideman *et al.*, 1981; Vincent *et al.*, 1983; Connelly *et al.*, 1989).

The mechanism by which CHIR 2279 elicits bradycardia in rats and guinea pigs cannot be gleaned from the data presented in this report, but should be the subject of further investigations of the pharmacology of the compound. Although it is possible that the peptoid acts directly on myocardial tissue, indirect mechanisms are equally plausible. The bradycardia caused by CHIR 2279 may, for example, be related to nonadrenergic activity of the compound. However, our extensive screening of the compound against a variety of receptors, enzymes and neurotransmitter uptake sites has failed to highlight any such interaction. Also, the bradycardia may be attributed to a central effect that induces sympathetic withdrawal. CHIR 2279 may gain access to the central nervous system via circumventricular organs or by diffusion

across the blood-brain barrier. We have found poor permeability of CHIR 2279 in an *in vitro* model for diffusion across the gastrointestinal epithelium (data not shown). Inasmuch as the permeability of drugs to gastrointestinal epithelia is similar to permeability across the blood-brain barrier (Benet *et al.*, 1993), this finding suggests that the onset of bradycardia that was observed shortly after i.v. administration (fig. 10) was not related to rapid diffusion of CHIR 2279 across the blood-brain barrier.

Dose-response relationships for MAP (fig. 11) suggested that guinea pigs were generally more sensitive than rats to MAP reduction by CHIR 2279; at 0.1 mg/kg in guinea pigs CHIR 2279 caused MAP to drop 10 to 20% for about 30 min, but in rats, 1 mg/kg had no effect on MAP. It was possible that the difference in the apparent sensitivity to CHIR 2279 in rats and guinea pigs was related to pharmacokinetic differences in the two species. (For example, the weaker responsiveness in rats might be attributed to a more rapid elimination of CHIR 2279 from the plasma.) To resolve this uncertainty, PK/PD analysis was performed in rats and guinea pigs, where changes in MAP were correlated with concentrations of CHIR 2279 in the plasma.

PK/PD analysis (table 8) showed that the systemic clearance rate for CHIR 2279 was approximately twice as fast in guinea pigs than rats (59.9 ml/min·kg in rats *vs.* 104 ml/min[chempt]kg in guinea pigs). As shown in figure 13C, the curve describing the relationship between concentration and effect was shifted to the left in guinea pigs relative to rats, and the corresponding EC₅₀ was 50% lower in guinea pigs (520 *vs.* 1170 ng/ml; table 8). Based on this result, CHIR 2279 was concluded to be twice as potent at reducing MAP in guinea pigs than rats. Differences in the *in vivo* potency of CHIR 2279 in rats and guinea pigs are possibly attributed to several factors that may have varied between rats and guinea pigs. These potential variables include biodistribution, protein binding, rates of metabolism and the generation of pharmacodynamically active metabolites. Although the data generated in our investigation are insufficient to address these possibilities, such factors should be the subject of further investigations of the pharmacology of CHIR 2279.

To summarize, competitive receptor-binding assays showed that CHIR 2279 was similar to prazosin in binding to α_{1A} , α_{1a} , α_{1b} and α_{1d} adrenoceptor subtypes with high affinity. However, the K_i values were generally 10 times weaker with CHIR 2279 than with prazosin. Functional bioassays showed that CHIR 2279 was approximately equipotent in antagonizing agonist-induced contractile responses with rat vas deferens (α_{1A}), canine prostate (α_{1A}), rat spleen (α_{1B}) and rat aorta (α_{1D}) and the pA₂ for CHIR 2279 indicated about a 10- to 100-fold lower *in vitro* potency than prazosin. In anesthetized dogs, CHIR 2279 antagonized the epinephrine-induced increase in IUP with a corresponding pseudo pA₂ of 6.86. In normotensive rats and guinea pigs, CHIR 2279 induced a dose-dependent decrease in MAP and heart rate, demonstrating an absence of the tachycardia commonly observed with other α_1 -blockers. PK/PD modeling showed that the i.v. systemic clearance rate of CHIR 2279 was twice as fast in guinea pigs as rats, and the *in vivo* potency for MAP reduction was twice as great in guinea pigs. Overall, the findings summarized in this report show that CHIR 2279 is a potent antagonist of α_1 -adrenoceptors with *in vitro* and *in vivo* pharmacological properties that are unique from known α_1 -pharmacophores.

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