JOURNAL OF
MEDICINAL
CHEMISTRY

Volume 38, Number 10 May 12, 1995

Communications to the Editor

Discovery of α_{1a}-Adrenergic Receptor Antagonists Based on the L-Type Ca^{2+} Channel Antagonist Niguldipine

John M. Wetzel,[†] Shou Wu Miao,[†] Carlos Forray,[‡] Laurence A. Borden,[‡,§] Theresa A. Branchek,[‡] and Charles Gluchowski*,[†]

Synaptic Pharmaceutical Corporation, Paramus, New Jersey 07652

Received February 3, 1995

α-Adrenergic receptors (α-ARs) serve key switching functions in both the central and peripheral nervous systems.[1] These proteins traverse the cell membrane with seven putative α helical segments. In response to changes in the extracellular concentration of the neurotransmitter norepinephrine, α-ARs modulate various intracellular biochemical processes via G proteins and effector systems. The involvement of α-ARs in the regulation of numerous physiological events makes them important targets for drug design. Potential therapeutic indications for α-adrenergic drugs include the treatment of benign prostatic hyperplasia (BPH),[2,3] cardiac arrhythmia,[4] glaucoma,[5] hypertension,[6] and pain.[7]

Prior to the mid-1970s, only one α-AR was known to exist. In the past 2 decades, new pharmacological tools and, more recently, molecular biological techniques have been used to identify and chemically characterize six α-ARs, which are designated α_{1a}, α_{1b}, α_{1d}, α_{2a}, α_{2b}, and α_{2c}.[1,8] The next challenge in this field will be to determine the distribution and physiological roles of each of these subtypes and to discover drugs that act specifically at individual subtypes. To this end, we have previously used cloned human α-AR assay systems to determine that the α_1-AR that mediates human prostatic smooth muscle tone has the pharmacological properties of the cloned α_{1a}-AR subtype (formerly referred to as the α_{1c}-AR subtype[8]).[9] We now report on the use of these cloned human receptor assay systems to discover antagonists that are specific for the human α_{1a}-AR. These compounds will be useful in receptor localization and functional characterization studies. They will also provide structural information of use to drug design efforts targeting various disorders, including BPH.

Cell membrane preparations from cell lines expressing the three cloned human α_1-AR genes, α_{1a}, α_{1b}, and α_{1d}, and the three cloned human α_2-AR genes, α_{2a}, α_{2b}, and α_{2c}, were used in radioligand displacement experiments as described previously.[9-11] The binding characteristics of structurally diverse, known α_1-AR antagonists were determined at these cloned human receptor subtypes by displacement of [^{3}H]prazosin at the α_1-ARs and [^{3}H]rauwolscine at the α_2-ARs.

Most of the known α_1-AR antagonists that were tested in the cloned human α-AR assay systems were found to have little or no selectivity for individual α_1-AR subtypes.[9] For example, structural analogs of prazosin, such as terazosin, doxazosin, alfuzosin, and abanoquil, were all found to bind nonselectively to all three human α_1-AR subtypes.[9,10] Some of these compounds have been used to treat BPH and found to cause undesired side effects, such as dizziness and asthenia.[2,3] These side effects may be due to cross-reaction of these compounds at the α_{1B} or α_{1D} receptor.

In contrast to the prazosin analogs, the 1,4-dihydropyridine (*S*)-(+)-niguldipine (*S*-(+)-**1**)[12] exhibited 340- and 630-fold selectivity in binding to the cloned human α_{1a}-AR relative to the α_{1b}-AR and α_{1d}-AR, respectively.[9] Moreover, this compound bound with high affinity (K_i = 0.2 nM) to the human α_{1a}-AR (see Table 1). (*S*)-(+)-Niguldipine also showed potent calcium channel blocking activity and has been investigated as an antihypertensive by Byk Gulden.[14] (*R*)-(−)-Niguldipine (*R*-(−)-**1**) was 29-fold less potent at the α_{1a}-AR than its enantiomer and also less subtype-selective. We designed and synthesized analogs of **1**, with the aim of achieving greater selectivity and affinity for the human α_{1a}-AR and reducing calcium channel affinity.

Results and Discussion

The affinity of 4-(nitrophenyl)-1,4-dihydropyridines for the L-type calcium channel is known to depend on

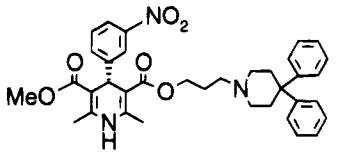
[†] Department of Chemistry.
[‡] Department of Pharmacology.
[§] Current address: Trophix Pharmaceuticals, Inc., 40 Cragwood Rd., South Plainfield, NJ 07080.

0022-2623/95/1838-1579$09.00/0

Table 1. Binding Affinities at Cloned Human α-ARs[a] and the Rat L-Type Calcium Channel[b]

	$K_i \pm$ SEM (nM)[c]						
	α_{1a}	α_{1b}	α_{1d}	α_{2a}	α_{2b}	α_{2c}	Ca^{2+}
S-(+)-**1**	0.16 ± 0.04	55 ± 8	100 ± 21	530 ± 100	420 ± 32	330 ± 48	4.6 ± 1
R-(−)-**1**	4.7 ± 1	56 ± 6	170 ± 28	900 ± 330	660 ± 81	450 ± 54	92 ± 24
8	0.32 ± 0.1	50 ± 5	210 ± 54	800 ± 65	570 ± 37	300 ± 52	250 ± 47
(±)-**9**	0.35 ± 0.06	220 ± 14	540 ± 48	1200 ± 85	800 ± 92	370 ± 81	540 ± 130
(+)-**9**	37 ± 7	410 ± 25	780 ± 79	1100 ± 150	860 ± 100	460 ± 81	370 ± 110
(−)-**9**	0.18 ± 0.02	180 ± 17	630 ± 43	1300 ± 210	780 ± 120	680 ± 73	670 ± 220
terazosin	6.9 ± 0.5	2.6 ± 0.04	3.7 ± 0.5	550 ± 69	26 ± 4	170 ± 23	>10 000
prazosin	0.27 ± 0.02	0.21 ± 0.02	0.30 ± 0.04	210 ± 43	13 ± 1	24 ± 3	>10 000

[a] Displacement of [^{3}H]prazosin from α_1-ARs (ref 9; incubation time 4 h) and [^{3}H]rauwolscine from α_2-ARs (ref 11). [b] Displacement of [^{3}H]nitrendipine from rat brain (ref 13). [c] Average of three to six independent determinations.



S-(+)-Niguldipine, **1**

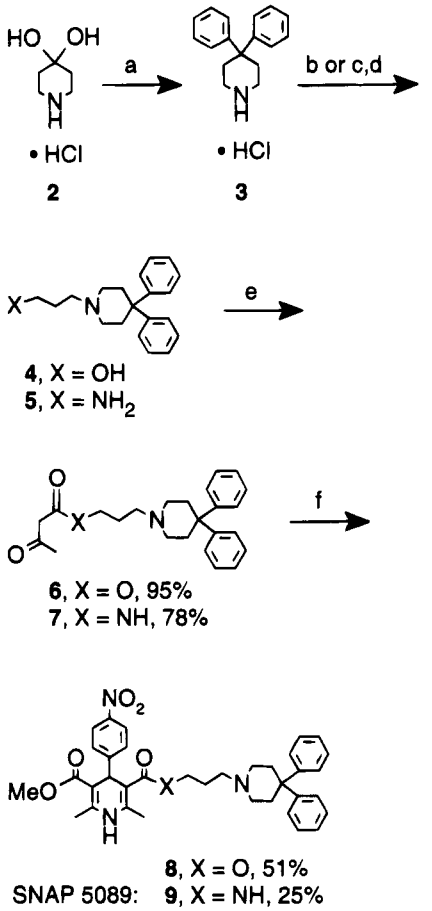
the position of the nitro group.[15] For example, in the nifedipine series (1,4-dihydro-2,6-dimethyl-4-(nitrophenyl)pyridine dicarboxylic acid dimethyl esters), the binding affinity of the 4-(4-nitrophenyl) analog to L-type calcium channels is 2−3 orders of magnitude lower than the affinities of the 4-(3-nitrophenyl) and 4-(2-nitrophenyl) analogs.[16] Therefore, in our search for analogs of **1** with reduced calcium channel affinity, we prepared the 4-(4-nitrophenyl) analog **8**[17] by the Hantzsch method[14] as shown in Scheme 1. We also examined the effect upon activity of an amide linkage attaching the 3-(4,4-diphenylpiperidin-1-yl)propyl side chain to the dihydropyridine nucleus, as in compound **9**. For pharmacological characterization, the monohydrochloride salts of **8** and **9** were used.

As expected, **8** exhibited a 54-fold reduction in potency relative to *S*-(+)-**1** as an antagonist of the L-type calcium channel (see Table 1). Notably, **8** maintained high affinity and subtype selectivity for the α_{1a}-AR. Amide **9**, which we designated SNAP 5089, showed an even more desirable binding profile than **8** (see Table 1). Compound **9** bound to the human α_{1a}-AR with K_i = 0.4 nM and exhibited selectivities of 630-, 1500-, and 1500-fold in binding to the α_{1a}-AR relative to the α_{1b}-AR, the α_{1d}-AR, and the L-type calcium channel, respectively. This compound was also >1000-fold selective in binding to the human α_{1a}-AR relative to the cloned human α_{2a}-, α_{2b}-, and α_{2c}-ARs.

To determine the abilities of compounds **8** and **9** to elicit functional (agonist) responses at cloned human α_1-ARs, changes in intracellular free calcium ion concentration were measured in transfected cells by microspectrofluorometry with the calcium-sensitive dye fura-2 (data not shown).[18] Neither **8** nor **9** was able to produce a functional response at a concentration of 1 μM at any of the three human α_1-ARs. However, at this concentration, both compounds were able to inhibit the intracellular calcium response induced by norepinephrine at a concentration of 10 μM. A 1 μM concentration of either **8** or **9** inhibited norepinephrine-induced liberation of intracellular calcium by 95%, 30%, or 20% in cells transfected with the α_{1a}-, α_{1b}-, or α_{1d}-AR, respectively.

The enantiomers of **9** were separated by HPLC on a chiral column.[19] The (−)-enantiomer proved to be more active at the α_{1a}-AR but less active at the L-type calcium channel than the (+)-enantiomer (see Table 1).

Scheme 1. Synthesis of **8** and **9**[a]



[a] (a) $AlCl_3$, benzene, 72%; (b) $Br(CH_2)_3OH$, K_2CO_3, 1,4-dioxane, 76%; (c) CH_2=CHCN, Et_3N, EtOH, 87%; (d) BH_3-THF, 66%; (e) diketene, toluene; (f) MeO_2CCH=$C(NH_2)CH_3$, 4-nitrobenzaldehyde, 2-propanol.

In summary, through the use of cloned human receptor assay systems, we have discovered the first subtype-selective antagonists for the human α_{1a}-AR. Compound **9** in particular shows high selectivity for the human α_{1a}-AR relative to the human α_{1b}-, α_{1d}-, α_{2a}-, α_{2b}-, and α_{2c}-ARs and the rat L-type calcium channel. Compounds **8** and **9** should prove exceedingly useful in the anatomical localization and functional characterization of human α_1-AR subtypes. Information gained through such studies will aid the discovery of new therapies for disorders where modulation of α-ARs proves to be beneficial. The effects of **8** and **9** in inhibiting α_{1A}-AR-mediated contraction of human prostate tissue *in vitro* will be reported in due course.

Acknowledgment. We are grateful to Dr. J. Paul Hieble of SmithKline Beecham Pharmaceuticals for communicating the recommendations of the IUPHAR Subcommittee on Adrenoceptor Nomenclature prior to publication. We acknowledge the technical assistance of Ms. Nancy Connerton and Ms. Debby Tambe in cell culture and membrane preparation and Mr. Boshan Li in radioligand displacement assays. We also thank Dr. Paul R. Hartig for helpful discussions and support.

Supplementary Material Available: Detailed synthetic procedures and spectroscopic and analytical data for **8** and **9** (6 pages). Ordering information is given on any current masthead page.

References

(1) Bylund, D. B.; Eikenberg, D. C.; Hieble, J. P.; Langer, S. Z.; Lefkowitz, R. J.; Minneman, K. P.; Molinoff, P. B.; Ruffolo, R. R., Jr.; Trendelenburg, U. IV. International Union of Pharmacology Nomenclature of Adrenoceptors. *Pharmacol. Rev.* **1994**, *46*, 121–136.

(2) Lepor, H. The Emerging Role of Alpha Antagonists in the Therapy of Benign Prostatic Hyperplasia. *J. Androl.* **1991**, *12*, 389–394 and references cited therein.

(3) Caine, M. Alpha-Adrenergic Blockers for the Treatment of Benign Prostatic Hyperplasia. *Urologic Clinics of North America* **1990**, *17*, 641–649 and references cited therein.

(4) Spiers, J. P.; Harron, D. W. G.; Wilson, R. Duration of Action and Effect on Baroreflex Function of the Anti-Arrhythmic Alpha 1 Antagonist UK 52,046. *J. Pharm. Pharmacol.* **1987**, *43*, 70–72.

(5) Serle, J. B.; Podos, S. M.; Abundo, G. P.; Ritch, R.; Coleman, A. L.; Panek, W. C.; Mantras, C. E. The Effect of Brimonidine Tartrate in Glaucoma Patients on Maximal Medical Therapy. *Invest. Opthalmol. Vis. Sci.* **1993**, *34*, 929.

(6) Gifford, R. W.; et al. The Fifth Report of the Joint National Committe on Detection, Evaluation, and Treatment of High Blood Pressure (JNC V). *Arch. Intern. Med.* **1993**, *153*, 154–183.

(7) Mendez, R.; Eisenach, J. C.; Kashtan, K. Epidural Clonidine Analgesia After Cesarean Section. *Anesthesiology* **1990**, *73*, 848–852.

(8) A new α_1-AR classification scheme similar to that proposed by Ford *et al.* (Ford, A. P. D. W.; Williams, T. J.; Blue, D. R.; Clarke, D. E. α_1-Adrenoceptor Classification: Sharpening Occam's Razor. *Trends Pharmacol. Sci.* **1994**, *15*, 167–170) was adopted at the August 1994 meeting of the International Union of Pharmacology (IUPHAR) in Montreal, Canada. The α_1-AR genes formerly known as $\alpha_{1a/d}$, α_{1b}, and α_{1c} were renamed α_{1d}, α_{1b}, and α_{1a}, respectively. This new naming system reflects the correspondence between the proteins encoded by the α_{1a} and α_{1b} genes (new IUPHAR nomenclature) and the receptors characterized by traditional pharmacological means as α_{1A} and α_{1B}, respectively, in the literature. Note that recombinant receptors and receptors characterized pharmacologically in tissues are distinguished by lowercase and uppercase subscripts, respectively.

(9) Forray, C.; Bard, J. A.; Wetzel, J. M.; Chiu, G.; Shapiro, E.; Tang, R.; Lepor, H.; Hartig, P. R.; Weinshank, R. L.; Branchek, T. A.; Gluchowski, C. The α_1-Adrenergic Receptor that Mediates Smooth Muscle Contraction in Human Prostate has the Pharmacological Properties of the Cloned Human α_{1c} Subtype. *Mol. Pharmacol.* **1994**, *45*, 703–708.

(10) Wetzel, J. M.; Miao, S. W.; Gluchowski, C.; Forray, C.; Vaysse, P. J.-J.; Borden, L. A.; Branchek, T. A.; Bard, J. A.; Weinshank, R. L.; Hartig, P. H. Binding and Intrinsic Activity Profiles of 4-Aminoquinazolines and 4-Aminoquinolines at Cloned Human Alpha Adrenergic Receptors. Presented at the 206th ACS National Meeting, Chicago, IL, Aug. 22–27, 1993; MEDI 53.

(11) Michel, M. C.; Kerker, J.; Branchek, T. A.; Forray, C. Selective Irreversible Binding of Chloroethylclonidine at α_1- and α_2-Adrenoceptor Subtypes. *Mol. Pharmacol.* **1993**, *44*, 1165–1170.

(12) Boer, R.; Grasseger, A.; Schudt, C.; Glossman, H. (+)-Niguldipine Binds with Very High Affinity to Ca^{2+} Channels and to a Subtype of α_1-Adrenoceptors. *Eur. J. Pharmacol., Mol. Pharmacol. Sect.* **1989**, *172*, 131–145.

(13) Bolger, G. T.; Gengo, P.; Klockowski, R.; Luchowski, E.; Siegel, H.; Janis, R. A.; Triggle, A. M.; Triggle, D. J. Characterization of Binding of the Ca^{2-} Channel Antagonist, [^{3}H]Nitrendipine, to Guinea-Pig Ileal Smooth Muscle. *J. Pharmacol. Exp. Ther.* **1983**, *225*, 291–309.

(14) Flockerzi, D.; Ulrich, W.-R. U.S. Patent 4,975,440, 1990.

(15) Loev, B.; Goodman, M. M.; Snader, K. M.; Tedeschi, R.; Macko, E. "Hantzsch-Type" Dihydropyridine Hypotensive Agents. 3. *J. Med. Chem.* **1974**, *17*, 956–965.

(16) Triggle, D. J.; Hawthorne, M.; Gopalakrishnan, M.; Minarini, A.; Avery, S.; Rutledge, A.; Bangalore, R.; Zheng, W. Synthetic Organic Ligands Active at Voltage-Gated Calcium Channels. *Ann. N.Y. Acad. Sci.* **1991**, *635*, 123–138.

(17) All new compounds gave satisfactory spectroscopic data. Final products gave satisfactory C, H, N analyses.

(18) Bush, A. B.; Borden, L. A.; Greene, L. A.; Maxfield, F. R. Nerve Growth Factor Potentiates Bradykinin-Induced Calcium Influx and Release in PC12 Cells. *J. Neurochem.* **1991**, *57*, 562–574.

(19) A Chiralpak AS column (20 × 250 mm, Daicel) was eluted with EtOH–hexane–diethylamine (20:80:0.1) with UV detection at 300 nm. The purified enantiomers, which were isolated in their free base forms, gave the following optical rotations: (−)-**9**, $[\alpha]_D$ = −50.9° ($CHCl_3$, 0.00865 g/mL); (+)-**9**, $[\alpha]_D$ = +52.5° ($CHCl_3$, 0.00615 g/mL).

JM950077B