mouse striatal slices was carried out in an identical fashion as described in an earlier publication.[12]

Acknowledgment. We thank the National Institute of Health (NIH Grant NS-17907) and the National Science Foundation (graduate fellowship for M.W.H.) for financial support of this work. We also thank Carol Settles for her assistance with the preparation of this manuscript.

Registry No. **5**, 15676-16-1; *cis*-**6**, 118894-81-8; *trans*-**6**, 118894-82-9; **7**, 118894-83-0; **8**, 118894-84-1; **9**, 118894-85-2; **10**, 579-75-9; **11**, 22117-85-7; **12**, 67833-50-5; **13**, 118894-86-3; **14**, 118894-87-4; **14** (sulfonyl chloride deriv.), 118894-88-5; **15**, 67304-97-6; **16**, 118894-89-6; 2-methoxy-5-(chlorosulfonyl)benzoic acid, 51904-91-7; 2,5-dichloroamylamine hydrochloride, 62922-45-6; benzoic acid, 65-85-0; 2-tetrahydrothiophenemethylamine, 83171-40-8.

A Minor Modification of Residue 1 in Potent Vasopressin Antagonists Dramatically Reduces Agonist Activity

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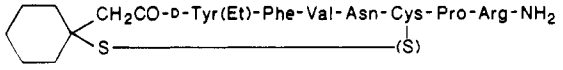
[1-(β,β-Pentamethylene-β-mercaptopropionic acid),2-(*O*-ethyl)-D-tyrosine,4-valine,9-desglycine]arginine-vasopressin (SK&F 101926, **1**), a potent in vivo and in vitro vasopressin V_2 receptor antagonist, was recently tested in human volunteers and shown to be a full antidiuretic agonist. A new animal model for vasopressin activity has been developed in dogs that duplicates the clinical agonist findings exhibited with SK&F 101926. In this model we have discovered that substitution of a *cis*-4′-methyl group on the Pmp moiety at residue 1 of vasopressin antagonists results in substantially reduced agonist activity compared to the unsubstituted molecule (SK&F 101926). The corresponding analogue with a *trans*-4′-methyl group exhibits more agonist activity than the cis molecule. These findings can be explained by viewing the biological activities of compounds such as **1** as the interaction of the vasopressin receptor with a number of discrete molecular entities, conformers of **1**, which present different pharmacophores. Models have been developed to assist in the understanding of these results.

Evaluation of a potent vasopressin V_2 receptor antagonist [1-(β,β-pentamethylene-β-mercaptopropionic acid),2-(*O*-ethyl)-D-tyrosine,4-valine,9-desglycine]arginine-vasopressin (SK&F 101926,[1] **1**)] in human volunteers

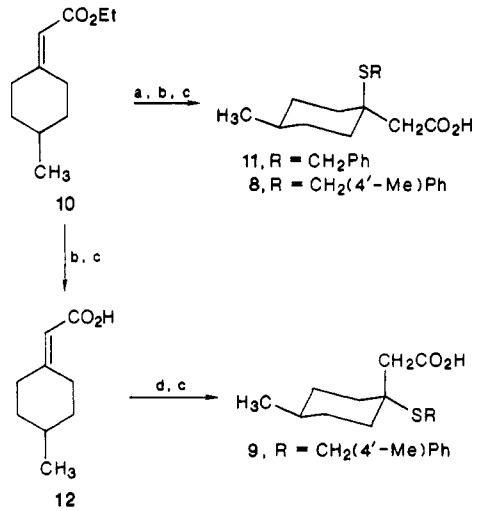


1

revealed that the molecule behaved as an antidiuretic agonist rather than an antagonist.[2] This result was unexpected since SK&F 101926 is (1) a potent in vivo antidiuretic hormone antagonist (aquaretic) in rat, dog, and squirrel monkey and (2) a potent in vitro V_2-receptor antagonist in rat, dog, squirrel monkey, and human renal medullary tissue preparations with no apparent agonist activity.[3] In an attempt to derive an animal model for the agonist activity observed in humans, we discovered that pretreatment of dogs with cyclooxygenase inhibitors such as indomethacin unmasks in vivo agonist activity of SK&F 101926.[4] We have subsequently applied the model to the determination of the structure–activity relationship (SAR) for partial agonism of V_2-receptor antagonists in dog. Of particular interest for study were those structural modifications that appeared to be essential for the initial conversion of agonists into antagonists.

One such modification was the pentamethylene-mercaptopropionic acid (Pmp) residue substituted at position 1 of vasopressin.[5] Early in our work with vasopressin antagonists we developed a synthesis of Pmp[6] that allowed us to prepare a wide variety of Pmp-modified peptides to test design hypotheses with regard to steric bulk and electronic factors.[7] In preliminary V_2-receptor assays the biological activity of vasopressin analogues containing these Pmp modifications proved generally unexceptional. However, when these peptides were evaluated in the indomethacin-petreated dog model for

Scheme I. Synthesis of 4′-MePmp Intermediates[a]



[a] (a) 0.1 equivalent of NaH, excess mercaptan, toluene; (b) K_2CO_3, water/MeOH; (c) HCl; (d) mercaptan, excess piperidine, reflux.

† Department of Pharmacology.
§ Department of Physical and Structural Chemistry.
‡ Department of Molecular Pharmacology.

(1) (a) Stassen, F. L.; Berkowitz, B. B.; Huffman, W. F.; Wiebelhaus, V. D.; Kinter, L. B. In *Diuretics: Chemistry, Pharmacology and Clinical Applications*; Puschett, J., Ed.; Elsevier: New York, 1984; p 64. (b) Manning, M.; Olma, A.; Klis, W.; Kolodziejczyk, A.; Nawrocka, E.; Misicka, A.; Seto, J.; Sawyer, W. *Nature (London)* **1984**, *308*, 652. (c) Huffman, W.; Moore, M. U.S. Patent 4 469 679, 1984.
(2) Dubb, J.; Allison, N.; Tatoian, D.; Blumberg, A.; Lee, K.; Stote, R. *Kidney Int.* **1987**, *31*, 267.
(3) Kinter, L. B.; Huffman, W. F.; Stassen, F. L. *Am. J. Physiol.* **1988**, *254* (*Renal Fluid Electrolyte Physiology 23*), 165.
(4) Albrightson, C.; Caldwell, N.; Brennan, K.; DePalma, D.; Kinter, L. B. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1987**, *46*, 1284.
(5) Manning, M.; Lammek, B.; Kolodziejczyk, A. M.; Seto, J.; Sawyer, W. H. *J. Med. Chem.* **1981**, *24*, 701.

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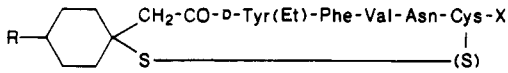
Table I. Activity of Vasopressin Antagonists



compd	R	X	rat: ED_{300},[a] μg/kg	pig: K_{Bind},[b] nM	human: K_i,[c] nm	dog K_i, nm	dog max U_{osm},[d] mOsm/kg
1	H	Pro-Arg-NH_2	15 (10)[e]	12 (8)	3.6 (17)	1.1 (4)	1625 ± 142 (7)
2	Me, cis	Pro-Arg-NH_2	45	19	nt	0.99	91 (2)
3	Me, trans	Pro-Arg-NH_2	22	100	nt	4.9	613 ± 346 (3)
4	H	Pro-Arg-Gly-NH_2	11 (9)[f]	12 (9)	4.6 (10)	1.0 (5)	1216 ± 423 (3)
5	Me, cis	Pro-Arg-Gly-NH_2	25 (3)	16 (3)	4.3 (3)	1.0 (3)	85 ± 9 (3)
6	H	Arg-D-Arg-NH_2	13.5 (5)	4.5 (4)	4.5 (4)	0.5 (5)	469 ± 221 (6)
7	Me, cis	Arg-D-Arg-NH_2	46 (3)	4.2 (2)	3.9	0.7	103 ± 7 (3)

[a] Dose required to lower urine osmolality to 300 mOsm/kg H_2O. [b] Measured by inhibition of binding of [^{3}H]LVP to renal medullary membrane preparation. [c] Inhibition of vasopressin-stimulated adenylate cyclase in renal medullary preparation. [d] Maximum urine osmolality with a dose of 100 μg/kg peptide; values are mean ±SE; indomethacin control has value of 111 ± 38 (6); AVP control (10 ng/kg) has value of 1281 ± 173 (3). [e] Number in parentheses is number of determinations. [f] ED_{300} determined in experiments in which control value for compound 1 was determined to be 9.2 μg/kg (see ref 1). nt = not tested.

agonist activity, we discovered that minor structural modifications about residue 1 could dramatically alter the partial agonist activity of V_2-receptor antagonists. In particular, we wish to describe the synthesis and biological activity of vasopressin V_2 receptor antagonists **2**, **3**, **5**, **7** (Table I) which contain *cis*- or *trans*-4′-MePmp (**8**, **9**) substitutions at residue 1.

Chemistry

Integral to the preparation of the desired peptide antagonist analogues was the synthesis of either *cis*- or *trans*-4′-MePmp (**8**, **9**). A recent publication from Lammek et al.[8] describes the use of 4′-MePmp in certain vasopressin V_1 receptor antagonists; however, no mention is made of the possibility of geometrical isomers. As depicted in Scheme I, Michael addition of either benzyl mercaptan or 4-methylbenzyl mercaptan to ethyl (4-methylcyclohexylidene)acetate (**10**) with catalytic sodium hydride as base,[6] followed by ester hydrolysis, affords 4′-MePmp where the major product is the isomer with the methyl group and the sulfur atom on the same side of the cyclohexane ring (arbitrarily defined as the cis isomer). The cis stereochemical relationship (axial sulfur atom) was determined by X-ray crystallography[9] of the benzyl mercaptan adduct **8**. When the Michael addition of mercaptan was carried out with the corresponding acid **12**, using excess piperidine as base,[10,11] we discovered that the trans adduct **9** was the principal product after fractional crystallization. The trans orientation between the methyl group and the sulfur atom (equatorial sulfur) in compound **9** was also confirmed via X-ray crystallography.[9] Incorporation of the modified Pmp derivatives into the final peptides followed conditions identical with those used for Pmp itself.[12]

Results and Discussion

The biological activities of the 4′-MePmp analogues (**2**, **3**, **5**, **7**) and their Pmp congeners (**1**, **4**, **6**) are listed in Table I. A direct comparison of the cis and trans geometrical isomers of 4′-MePmp was made in the family of antagonists which contained Pro-Arg-NH_2 as the carboxy-terminal portion of the molecule (compounds **1**–**3**).[1] The *cis*-4′-MePmp analogue **2** displayed affinity for both the pig and dog renal V_2 receptor, as determined by pig K_{Bind} and dog K_i, which was similar to that of the unsubstituted Pmp compound **1**. The analogue containing the *trans*-4′-MePmp substitution, compound **3**, appears to have diminished affinity for the pig and dog receptors. When compounds **1**–**3** were evaluated for in vivo V_2-receptor antagonism in rat, their diuretic (aquaretic) activity, as determined by the dose required to lower urine osmolality to serum levels, was indistinguishable. This apparent discrepancy between in vivo and in vitro results could reflect differences in pharmacokinetic properties or could be indicative of a species difference at the V_2 receptor, a result that is not without considerable precedent in vasopressin antagonists.[3]

Compounds **1**–**3** were also compared in the newly developed dog agonist model. The trans isomer **3** exhibited variable agonist activity. However, to our surprise, the *cis*-4′-MePmp-containing analogue **2** demonstrated considerably less agonist activity than the corresponding unsubstituted analogue **1**. In order to test the general nature of this observation, we examined the effect of the *cis*-4′-MePmp substitution in two additional series of antagonists in which the tripeptide tail was either the naturally occurring Pro-Arg-Gly-NH_2 (compounds **4**[13] and **5**) or the potent dibasic Arg-D-Arg-NH_2 replacement (compounds **6**[14] and **7**). Similar results were obtained with both series of molecules as indicated in Table I. In vitro activity in rat, pig, or dog and in vivo activity in rat was virtually identical for the Pmp and *cis*-4′-MePmp analogues. As observed before, agonist activity was reduced in the *cis*-

(6) Yim, N. C. F.; Huffman, W. F. *Int. J. Peptide Protein Res.* **1983**, *21*, 568.
(7) Yim, N. C. F.; Ali, F. A.; Bryan, W. M.; Huffman, W. F.; Moore, M.; Silvestri, J.; Erickson, R.; Heckman, G.; Kinter, L.; McDonald, J.; Schmidt, D.; Shue, D.; Stassen, F.; Stefankiewicz, J.; Sulat, L., Wang, S.-S. In *Peptides: Structure and Function, Proceedings of the Ninth American Peptide Symposium*; Deber, C. M., Hruby, V. J., Kopple, K. D., Eds.; Pierce Chemical Co.: Rockford, IL, 1985; p 615.
(8) Lammek, B.; Rekowski, P.; Kupryszewski, G.; Merlin, P.; Ragnarsson, U. *J. Med. Chem.* **1988**, *31*, 603.
(9) Eggleston, D. S.; Yim, N. C. F.; Silvestri, J. S.; Bryan, H.; Huffman, W. F. *Acta Crystallogr.*, in press.
(10) Vavrek, R. J.; Ferger, M. F.; Ashled, A. G.; Rich, D. H.; Bloomquist, A. T.; du Vigneaud, V. *J. Med. Chem.* **1972**, *15*, 123.
(11) Yim, N. C. F.; Moore, M. L.; Huffman, W. F.; Bryan, H. G.; Chang, H.-L.; Kinter, L. B.; Edwards, R.; Stassen, F. L.; Schmidt, D.; Heckman, G. *J. Med. Chem.* **1986**, *29*, 2425.
(12) Ali, F. E.-F.; Bryan, W.; Chang, H.-L; Huffman, W. F.; Moore, M. L.; Heckman, G.; Kinter, L. B.; McDonald, J.; Schmidt, D.; Shue, D.; Stassen, F. L. *J. Med. Chem.* **1986**, *29*, 984.
(13) Manning, M.; Olma, A.; Klis, W. A.; Kolodziejczyk, A. M.; Seto, J.; Sawyer, W. H. *J. Med. Chem.* **1982**, *25*, 45.
(14) Ali, F. E.-F.; Chang, H.-L.; Huffman, W. F.; Heckman, G.; Kinter, L. B.; Weidley, E. F.; Edwards, R.; Schmidt, D.; Ashton-Shue, D.; Stassen, F. L. *J. Med. Chem.* **1987**, *30*, 2291.

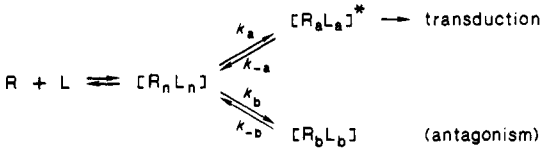
4'-MePmp analogue compared to its unsubstituted congener.

It is important to question how exchange of a methyl for a hydrogen could result in such a significant reduction in agonist activity. Preliminary conformational studies of compounds **1–3** using NMR spectroscopy indicate only very subtle differences between the molecules. No NOEs were observed between the 4'-methyl hydrogens and any hydrogens in the cyclic hexapeptide ring.[15] Additionally, comparison of the CD spectra of peptides **4** and **5** reveals no obvious differences in solution conformations.[16] Thus, substitution of *cis*- or *trans*-4'-methyl does not appear to have any effect on the ensemble of solution conformations and the differences in partial agonist activity are best interpreted as the result of intermolecular steric or electronic effects.

For visualizing the interaction of the vasopressin receptor with ligands such as antagonist **1**, we favor the mode depicted in Scheme II. Contributing to the selection of this model were two important observations regarding V_2-receptor antagonists. We have found striking differences between SAR of vasopressin agonists and antagonists, which led to the proposal that V_2-receptor antagonists bind quite differently to the vasopressin receptor than agonists and present a distinct antagonist pharmacophore.[17] Secondly, it is clear from low-temperature NMR studies that vasopressin antagonists like **1** are rapidly exchanging backbone conformations in solution at room temperature.[18] Thus, the biological activities of compounds such as **1** might best be viewed as the interaction of the receptor of interest with a number of discrete molecular entities, conformers of **1**, which present different pharmacophores.[19]

The model in Scheme II implies that changes in partial agonist activity could be the result of alterations in affinity for *either* the agonist or antagonist states; i.e. reduced partial agonist activity could result from decreased affinity

Scheme II. Model for Peptide Ligand–Receptor Interactions[a]



[a] R = receptor in various conformational states; R_a represents conformations of receptor that in combination with hormone or ligand result in demonstrable agonist signal transduction, R_b represents antagonist states. L = ligand in various conformational states where L_a represents conformations that display an agonist pharmacophore and initiate transduction and L_b represents conformations that display an antagonist pharmacophore.

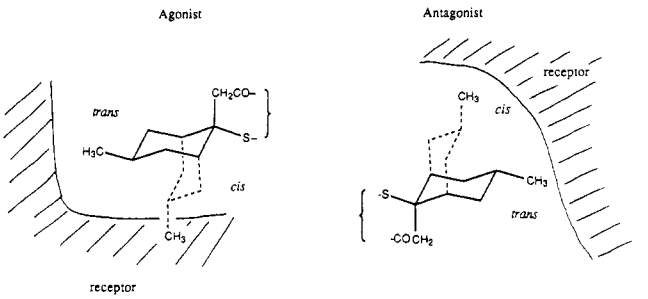
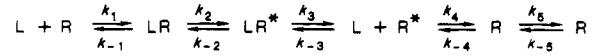


Figure 1. Model for pharmacophore interaction about residue 1 in vasopressin agonists and antagonists. The model has been presented in such a way as to emphasize the experimental findings[17] which indicate that agonists and antagonists bind differently to the vasopressin receptor.

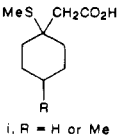
for the agonist state or increased affinity for the antagonist state.[20] In the case of vasopressin antagonists, the affinity of the ligand for the antagonist state can be approximated by the K_i which is a measure of the potency of any particular analogue to inhibit vasopressin-stimulated adenylate cyclase. The maximum osmolality achievable in the indomethacin-pretreated dog model serves as a measure of intrinsic agonist potential; we do not have a sensitive in vitro method to measure the affinity of molecules for the agonist state. Since the dog K_i's for the appropriate pairs of Pmp and *cis*-4'-MePmp analogues (**1–2, 4–5, 6–7**) are indistinguishable (Table I), the reduced partial agonist activity observed for the latter molecules should therefore not be the result of better affinity for the antagonist state. It would then follow that substitution of a 4'-hydrogen with a *cis*-4'-methyl group results in a reduced propensity for the analogue to adopt the agonist $[R_aL_a]$ pharmacophore.

Molecular mechanics calculations[21] of simple substituted

(15) Kopple, K.; DeBrosse, C. Unpublished results.
(16) Holl, W.; Webb, R. L. Unpublished results.
(17) Moore, M. L.; Huffman, W. F. In *Peptides: Chemistry and Biology: Proceedings of the Tenth American Peptide Symposium*; Marshall, G. R., Ed.; ESCOM: Leiden, 1988; p 438.
(18) DeBrosse, C. W.; Hempel, J. C.; Kopple, K. D. In *Peptides: Chemistry and Biology, Proceedings of the Tenth American Peptide Symposium*; Marshall, G. R., Ed.; ESCOM: Leiden, 1988; p 68.
(19) The model in Scheme II, which shares aspects of the dual receptor (see Ariens, E. J.; Beld, A. J.; Rodrigues de Miranda, J. F.; Simonis, A. M. In *The Receptors*, Volume 1; O'Brien, R. D., Ed.; Plenum Press: New York, 1979; Chapter 2) and the Macromolecular Perturbation Theory [Belleau, B. *J. Med. Chem.* **1964**, *7*, 776] models, describes the interaction in terms of the ligand and receptor both undergoing a succession of conformational changes that could be the result of factors such as induced fit, alterations in the membrane environment, etc. (R_nL_n). For a ligand such as **1**, which has a number of different conformations available to it with similar overall energy, the interaction with receptor produces a unique set of different "conformer-receptor" molecules. Ultimately these interactions separate into two groups, those that transduce agonist signal and those that do not. It is clear that a series of equilibrium states for each conformer–receptor interaction would most likely occur between R + L and the final transduction or lowest energy antagonist state. However, for the sake of simplicity, the model in Scheme II has been simplified to highlight one path leading to agonist pharmacophores $[R_aL_a]$ and another to antagonist pharmacophores $[R_bL_b]$ and assumes that the key equilibrium steps are those which occur at the point of divergence between agonist and antagonist states. Partial agonist activity could then be viewed in terms of k_a vs k_b/k_{-b} (or k_a/k_{-a} vs k_b/k_{-b} if the amount of transduction is related to length of time in $[R_aL_a]$).
(20) More traditionally, the interactions between ligands and receptors are viewed as shown below (from Carman-Krzan, M.



Progress in Medicinal Chemistry; Ellis, G. P., West, G. B., Eds.; Elsevier: Amsterdam, 1986; Vol. 23, pp 41–89). Agonists differ from antagonists in that they are able to form the activated complex LR*; partial agonists are defined as molecules for which $k_2 \simeq k_1$. This model differs from the model presented in Scheme II in that it is not theoretically possible to have an antagonist with less partial agonist activity solely as the result of improved affinity for the antagonist (LR) state.
(21) Molecular mechanics calculations were made with the MM2 force field (Model Version 1.2, W. C. Still, Columbia University) on the substituted cyclohexylacetic acid **i** (D. Chodosh, unpublished results).



i, R = H or Me

cyclohexylacetic acid analogues of 4'-MePmp indicate that the preferred orientations are consistent with the structures derived by X-ray crystallography. In the case of the *cis*-4'-MePmp derivative, the conformer with an equatorial methyl and axial sulfur is favored by 2.5 kcal/mol over the axial methyl, equatorial sulfur conformation. For the *trans*-4'-MePmp, the diequatorial relationship between the methyl and sulfur atoms is favored over the diaxial conformer by 1.0 kcal/mol. In the unsubstituted Pmp molecule, the axial orientation of sulfur is the lower energy conformation by 0.8 kcal/mol. Assuming these conformational differences are equally weighted in the conformations of the entire peptide, the hypothetical model illustrated in Figure 1 can be used to explain the partial agonist results. The basis of the model is the difference in the proximity of the ligand and receptor about position 1 in the pharmacophores for agonists and antagonists. The *cis*-4'-MePmp analogues (e.g. **3**) cannot adopt an agonist pharmacophore due to an unfavorable steric interaction between the receptor and the *cis*-4'-methyl group. This same limiting interaction does not occur in the case of the unsubstituted Pmp analogue **1**, which can present a sufficient agonist pharmacophore to initiate signal transduction. However, in the case of antagonist pharmacophore presentation, proper orientation of the key binding elements in the *cis*-4'-methyl analogues positions the molecules in such a way as to place the methyl group in a sterically allowed region. The *trans*-4'-MePmp analogue is accommodated somewhat in both the agonist and antagonist pharmacophores; however, its absolute affinity for either state is less than the affinity of the cis isomer for the antagonist state.

Conclusion

The results reported here indicate that subtle differences in the structure of vasopressin antagonists can afford major changes in agonist activity. A model to rationalize these unexpected findings is presented. Due to the complexities of conformational analysis of molecules such as vasopressin, the model is simplistic; nonetheless, it is consistent with the observed results. Continued hypothesis testing and model refinement should further delineate the differences between the agonist and antagonist pharmacophores for ligands at the vasopressin V_2 receptor.

Experimental Section

Melting points were determined on a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Proton NMR were obtained on a Varian EM-390 spectrometer at 90 MHz using tetramethylsilane (TMS) as an internal standard. Elemental analyses were performed on a Perkin-Elmer 240 apparatus by the Analytical Chemistry Department of Smith Kline & French Laboratories. Where analyses are reported by symbols of elements, results were within ±0.4% of the calculated values. Fast atom bombardment (FAB) mass spectrometry was performed by the Physical and Structural Chemistry Department of Smith Kline & French Laboratories on a VG ZAB high-resolution spectrometer. Amino acid analyses were performed on a Kontron Liquimat III amino acid analyzer. Protected amino acids were obtained from commercial sources with the exception of Boc-D-Tyr(Et), which was prepared by a literature method.[22] Benzhydrylamine resin (BHA resin, 1% cross-linked S-DVB, 200–400 mesh, ≃1.0 mequiv of N/g of resin) was made according to Bryan.[23] XAD-2 (polystyrene resin) was purchased from Mallinckrodt. Solvents and reagents were reagent grade. Methylene chloride (CH_2Cl_2) was HPLC grade and dimethylforamide (DMF) was stored over 4-Å molecular sieves and filtered prior to use. The purity of the peptides was routinely checked by HPLC with an Altex Ultrasphere 5μ ODS column (4.5 mm × 25 cm) with UV detection at 220 nm and thin-layer chromatography (TLC) on EM silica gel plates with visualization with 10% bleach/1% KI–starch. Common TLC solvents are 1-butanol/ethyl acetate/acetic acid/water (BEAW) and 1-butanol/acetic acid/water (BAW). For preparative HPLC a 10 mm × 25 cm Altex Ultrasphere 5μ ODS column was used. For analytical HPLC a linear gradient of water/acetonitrile containing 0.1% trifluoroacetic acid (TFA) was used (80:20 to 50:50 over 20 min at 1.5 mL/min). For amino acid analyses, peptides were hydrolyzed in HCl/TFA (2:1) containing 0.005% w/v phenol at 160 °C for 1 h.

***cis*-4-Methyl-1-[[(4-methylphenyl)methyl]thio]cyclohexaneacetic Acid (8) and *cis*-4-Methyl-1-[(phenylmethyl)thio]cyclohexaneacetic Acid (11).** The method was analogous to that described by Yim and Huffman.[6] Under argon, sodium hydride (60% oil dispersion, 220 mg, 2.75 mmol) was added to dry (molecular sieves) toluene (32.5 mL). Then *p*-methylbenzyl mercaptan (4.1 g, 27.5 mmol) was added dropwise and the reaction mixture was stirred for 0.5 h. Ethyl (4-methylcyclohexylidene)acetate (**10**)[6] (5.0 g, 27.5 mmol) was dissolved in toluene (32.5 mL) and DMF (1.5 mL) and this was added dropwise over a period of 15 min. Upon completion of addition, the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was poured onto ice water (500 mL), acidified to pH 2.0 with 3 N HCl, and extracted with diethyl ether (3 × 150 mL). The combined organic extracts were washed with brine (2 × 100 mL), dried ($MgSO_4$), filtered, and evaporated at reduced pressure to yield 8.25 g of ester (94%): ^{1}H NMR ($CDCl_3$) δ 7.35–7.05 (q, 4 H), 4.16–3.9 (q, 2 H), 3.7 and 3.55 (s, 2 H, SCH_2, 20:80, trans:cis), 2.65 and 2.51 (s, 2 H, CH_2CO_2Et, 20:80, trans:cis), 2.26 (s, 3 H), 2.1–1.1 (m, 8 H), 0.8 (d, 3 H); the signals for the cis and trans isomers were consistent with those obtained from the purified isomers as established by X-ray crystallography.

The above ester (6.6 g, 20.6 mmol) was dissolved in 170 mL of a solution containing K_2CO_3 (50 g) dissolved in 400 mL of 25% aqueous methanol and heated at reflux for 18 h. The methanol was removed under vacuum. The residue was diluted with water (50 mL) and washed with a solution of 1:1 (v/v) ether/hexane (3 × 50 mL). The aqueous solution was then acidified at 0 °C to pH 2.0 with 3 N HCl and extracted with diethyl ether (3 × 50 mL). The extracts were combined, washed with brine (2 × 25 mL), and dried ($MgSO_4$) to yield 3.0 g of the crude acid. The product was recrystallized from hexane to yield 1.14 g (19%) of pure cis isomer **8**: mp 92–94 °C; ^{1}H NMR ($CDCl_3$) δ 7.15 (q, 4 H), 3.6 (s, 2 H), 2.6 (s, 2 H), 2.3 (s, 3 H), 2.2–19 (m, 2 H), 1.8–14 (m, 4 H), 1.3–1.1 (m, 2 H), 0.9 (d, 3 H). Anal. ($C_{17}H_{24}O_2S$) C, H.

By the same procedure on a 50-mmol scale, using benzyl mercaptan in place of *p*-methylbenzyl mercaptan, crude *cis*-4-methyl-1-[(phenylmethyl)thio]cyclohexaneacetic acid (**11**) was obtained (39%). Recrystallization from hexane afforded pure **11** (17%), mp 75–76 °C. Anal. ($C_{16}H_{22}O_2S$) C, H.

***trans*-4-Methyl-1-[[(4-methylphenyl)methyl]thio]cyclohexaneacetic Acid (9).** Ethyl (4-methylcyclohexylidene)acetate (**10**)[6] (10.0 g, 55 mmol) was dissolved in 440 mL of a solution containing K_2CO_3 (100 g) dissolved in 800 mL of 25% aqueous methanol and refluxed for 18 h. The methanol was removed under vacuum and the residue was diluted with water (300 mL) and washed extensively with a solution of 1:1 (v/v) ether/hexane. The aqueous solution was then acidified to pH 2.0 with 3 N HCl at 0 °C and extracted with diethyl ether (3 × 100 mL). The ether extracts were combined, washed thoroughly with brine, dried ($MgSO_4$), filtered, and evaporated at reduced pressure. The residue was recrystallized from hexane to yield 7.3 g (45%) of pure acid **12**: MS *m/z* 155 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 5.66 (s, 1 H), 3.8–3.5 (m, 1 H), 2.4–2.1 (m, 2 H), 2.0–1.5 (m, 4 H), 1.3–1.1 (m, 2 H), 1.9 (d, 3 H). Anal. ($C_9H_{14}O_2$) C, H.

The above acid (500 mg, 3.25 mmol), piperidine (560 μL, 6.5 mmol), and *p*-methylbenzyl mercaptan (630 μL, 4.9 mmol) were dissolved in toluene (1 mL) and heated at reflux under argon for 18 h. The solution was diluted to 25 mL total volume with ethyl acetate and washed with 1 N HCl (2 × 10 mL), water (1 × 15 mL), and brine. The organic solution was dried ($MgSO_4$), filtered, and evaporated at reduced pressure to yield 863 mg of crude acid (91%). The product was recrystallized from hexane to yield 276

(22) Mendelson, W. L.; Tickner, A. M.; Lantos, I. *J. Org. Chem.* **1983**, *48*, 4127.
(23) Bryan, W. *J. Org. Chem.* **1986**, *51*, 3371.

mg (29%) of the pure trans isomer **9**: mp 132–134 °C; ^{1}H NMR ($CDCl_3$) δ 7.15 (q, 4 H), 3.8 (s, 2 H), 2.75 (s, 2 H), 2.3 (s, 3 H), 2.2–1.9 (m, 2 H), 1.8–1.4 (m, 4 H), 1.3–1.1 (m, 2 H), 0.9 (d, 3 H). Anal. ($C_{17}H_{24}O_2S$) C, H.

General Peptide Synthesis. The peptides were synthesized on 0.5 mmol of BHA resin. All couplings were performed with 3 equiv of the Boc-protected amino acid (including the *cis*- and *trans*-4′-MePmp derivatives) with 3 equiv of both 1,3-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) in a solution (1:2 v/v) of CH_2Cl_2 and DMF for 2–18 h. Couplings and deprotections were monitored by using the Kaiser ninhydrin test.[24] The BOC group was removed with a single 20-min treatment of 50% TFA in CH_2Cl_2 (~10 mL/g of peptide resin). The resulting amine TFA salt was neutralized with 3 × 2 min treatments of 7% diisopropylethylamine (DIEA) in CH_2Cl_2 (~10 mL/g of peptide resin). Protected peptide resins were cleaved and deprotected with anhydrous hydrogen fluoride (HF) with 10% anisole at 0 °C for 1 h. The HF was removed in vacuo at 0 °C and the resin was washed with diethyl ether and the peptide extracted into 2 L of degassed, distilled water using DMF, water, and TFA. The pH was adjusted to 7.2 with ammonium hydroxide. A solution of 0.01 M potassium ferricyanide was then added dropwise with stirring until a faint yellow color persisted for 15 min. The pH was then adjusted to 4.5 with acetic acid and the entire solution was passed through a XAD-2 column (5 × 25 cm). The column was washed with water (~100 mL) and the peptide eluted with 50% acetonitrile 50% water containing 0.1% TFA. The eluant was evaporated and the peptide lyophilized from HOAc.

[*cis*-4′-MePmp^1,D-Tyr(Et)^2,Val^4,desGly^9]AVP (2). The crude peptide was purified by preparative HPLC isocratically with 60:40 water/acetonitrile containing 0.1% TFA at 4.0 mL/min to yield 13 mg of pure peptide **2**: HPLC k' = 11.80; TLC (BEAW, 1:1:1:1) R_f = 0.68; FAB mass spectrum, m/z 1092 $(M + H)^+$; amino acid analysis, Asp 1.0, Val 0.85, Tyr 0.90, Phe 0.83, Arg 0.98, Pro 1.39, Cys 0.88.

[*trans*-4′-MePmp^1,D-Tyr(Et)^2,Val^4,desGly^9]AVP (3). The peptide was purified by preparative HPLC using 65:35 water/acetonitrile containing 0.1% TFA at 4.0 mL/min to yield 60 mg of pure peptide: HPLC k' = 10.82; TLC (BEAW, 1:1:1:1) R_f = 0.62; FAB mass spectrum, m/z 1092 $(M + H)^+$; amino acid analysis, Asp 1.0, Pro 1.27, Cys 0.89, Val 1.00, Tyr 0.98, Phe 0.98, Arg 1.12.

[*cis*-4′-MePmp^1,D-Tyr(Et)^2,Val^4]AVP (5). The crude peptide was purified by countercurrent distribution with 1-butanol–acetic acid/water (4:1:5) followed by gel filtration on Sephadex G-15 (0.2 N HOAc): HPLC k' = 7.6; TLC (BAW, 4:1:5) R_f = 0.63; FAB mass spectrum, m/z 1050 $(M + H)^+$; amino acid analysis, Asp 0.92 Pro 1.02, Gly 1.00, Cys 0.56, Val 0.96, Tyr 0.57, Phe 0.92, Arg 0.88.

[*cis*-4′-MePmp^1,D-Tyr(Et)^2,Val^4,Arg^7,D-Arg^8,desGly^9]AVP (7). The crude peptide (246 mg) was purified by countercurrent distribution using the system BAW (4:1:5) to yield 51 mg of partially purified peptide. This was further purified by gel filtration on Sephadex G-15 (1% HOAc) to afford 35 mg of pure peptide: HPLC (Hamilton PRP-1, CH_3CN/H_2O/0.1% TFA, 20–50% CH_3CN, 15 min) k' = 4.83; TLC (BEAW, 1:1:1:1) R_f = 0.63; TLC (BAW 1:1:1) R_f = 0.59; FAB mass spectrum, m/z 1152 $(M + H)^+$; amino acid analysis, Asp 1.00, Val 1.04, Tyr 0.91, Phe 0.97, Arg 1.93.

Bioassy Methods. Vasopressin V_2 receptor binding was measured by competition with tritiated lysine vasopressin in a pig renal medullary preparation as described previously[25] and is expressed as a binding constant (K_{Bind}). Inhibition of vasopressin-sensitive adenylate cyclase was measured in canine or human renal medullary tissue as described previously[26] and is expressed as an inhibition constant (K_i). The in vivo evaluation for antagonist activity in the hydropenic rat model has been described in detail.[27] Potency is expressed as the effective dose (ED_{300}, μg/kg) required to reduce Uosm from hydropenic levels to plasma osmolality levels (300 mOsm/kg H_2O). A preliminary description of the indomethacin-pretreated water-loaded dog model has been made published.[4] Briefly, adult female mongrel dogs were trained to lie, lightly restrained, on their backs. Food but not water was withheld for 18 h prior to each experiment. The dogs were hydrated with tap water (5% body weight, orally by gavage) 60 min prior to the insertion of a urinary bladder catheter. The bladder was cleared with air and emptied via palpation, and urine flow (mL/min) and osmolality (Uosm, mOsm/kg H_2) were determined at 10-min intervals. Urine and plasma osmolality were measured by using the freezing point depression technique. An indwelling catheter was inserted into the saphenous vein from which blood is taken and through which 3% dextrose was administered at 5 mL/min. That infusion rate was maintained until the urine osmolality was stabilized below 70 mOsm/kg H_2O (baseline water diuresis) at which time a steady state of hydration was maintained by adjusting the rate of dextrose infusion to match the urine output of the preceding 10-min collection period. Baseline water diuresis was maintained for 20 min prior to the administration of test compounds or cyclooxygenase inhibitors. When administered, a loading dose and infusion of indomethacin (2 mg/kg + 3 mg/kg per h) were delivered via the dextrose infusion line. Plasma osmolality was determined prior to dextrose infusion and at the conclusion of the experiment. After 20 min of baseline water diuresis and 20 min of indomethacin treatment, a single bolus intravenous injection of the test compound (100 μg/kg) or vehicle was administered. The agonist activity of these compounds was determined by their effect on urine flow and osmolality as measured at 10-min intervals for 3 h.

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(24) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.

(25) Stassen, F. L.; Erickson, R. L.; Huffman, W. F.; Stefankiewicz, J.; Sulat, L.; Wiebelhaus, V. D. *J. Pharmacol. Exp. Ther.* **1982**, *223*, 50.

(26) Stassen, F. L.; Heckman, G. D.; Schmidt, D. B.; Stefankiewicz, J.; Sulat, L.; Huffman, W. F.; Moore, M. L.; Kinter, L. B. *Vasopressin*; Schrier, R. W., Ed.; Raven Press: New York, 1985; p 145.

(27) Kinter, L.; Huffman, W.; Wiebelhaus, V.; Stassen, F. *Diuretics: Chemistry, Pharmacology and Clinical Applications*; Puschett, J., Ed.; Elsevier: New York, 1984; p 72.