[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),4-valine,-8-D-arginine]vasopressin, a Potent and Selective Inhibitor of the Vasopressor Response to Arginine-vasopressin

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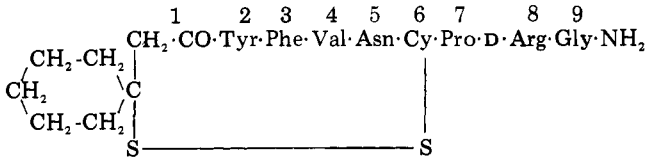
As part of a program in which we are attempting the design and synthesis of an antagonist of the antidiuretic response to arginine-vasopressin (AVP) [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine,8-D-arginine]vasopressin [d(CH_2)$_5$VDAVP] was synthesized and assayed for antidiuretic, vasopressor, and oxytocic activities. The required protected intermediate was synthesized by a combination of solid-phase synthesis and an [8 + 1] coupling in solution. d(CH_2)$_5$VDAVP has an antidiuretic potency of 0.10 ± 0.02 unit/mg, less than $^1/_{10\,000}$ that of its parent [deamino,4-valine,8-D-arginine]vasopressin (dVDAVP). d(CH_2)$_5$VDAVP is a specific antagonist of the vasopressor responses to AVP. It has an antivasopressor pA_2 value of 7.68 ± 0.05 when tested against AVP. It is also an antagonist of the in vitro oxytocic response to oxytocin (pA_2 value = 6.62 ± 0.07). With its negligible antidiuretic activity, absence of oxytocic activity, and its potent and specific ability to antagonize the vasopressor effects of AVP, d(CH_2)$_5$VDAVP is one of the most potent and selective vasopressor antagonists reported to date. It should thus be a useful tool with which to probe the possible role(s) that AVP may play in cardiovascular regulation under normal and pathological conditions.

A previous communication from these laboratories reported the synthesis and some pharmacological properties of [1-deaminopenicillamine,4-valine,8-D-arginine]vasopressin (dPVDAVP).[1] We prepared this peptide in order to investigate the possibility that modifying the potent and specific antidiuretic agent [1-deamino,4-valine,8-D-arginine]vasopressin (dVDAVP)[2-4] by incorporating a β,β-dialkyl substitution at position 1 might lead to a peptide antagonist of the antidiuretic response to AVP. It was reasoned that the productive recognition of dVDAVP by the receptor(s) mediating the antidiuretic response, implicit in the peptide's potency and specificity, might be rendered nonproductive by the change in structure at position 1. Such modifications have been reported to give rise to analogues of oxytocin,[5] deamino-oxytocin,[5-7] and deamino-lysine-vasopressin[8] exhibiting a variety of antihormonal properties.

While not an anti-antidiuretic agent, the peptide dPVDAVP proved of interest in that it retained only $^1/_{10}$th of the antidiuretic activity of dVDAVP and showed antivasopressor activity enhanced sixfold over that of dVDAVP. Thus dPVDAVP was a potentially useful pharmacological tool, due to its capacity to antagonize the vasopressor effect of AVP.

With the goal of obtaining an anti-antidiuretic peptide derived from dVDAVP still in mind, it was noted that in a series of antioxytocic and antivasopressor analogues of oxytocin, the inhibitory potencies varied markedly with variation in the bulk/hydrophobicity of the β-carbon substituent at position 1. The most potent antioxytocic agent of this series was [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)]oxytocin.[7] Considering that the low antidiuretic activity of dPVDAVP compared to that of dVDAVP may not be inconsistent with the original conjecture that the productive interaction of dVDAVP with antidiuretic receptor(s) might be impaired by the introduction of the dimethyl group at the β carbon in position 1, it thus seemed worthwhile to examine [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine,8-D-arginine]vasopressin [d(CH_2)$_5$VDAVP] for antihormonal properties. It has the following structure.



Its synthesis and pharmacological evaluation are reported here.

The octapeptide derivative Boc-Tyr(Bzl)-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2 was prepared using solid-phase methodology[9-12] as previously described.[13] The product of the removal of the N-terminal protecting group was coupled with β-(*S*-benzylmercapto)-β,β-cyclopentamethylenepropionic acid[7] by a dicyclohexylcarbodiimide (DCCI)–*N*-hydroxybenzotriazole (HOBt) preactivation method[14,15] to yield the immediate precursor of the required disulfide-bridged cyclic peptide. All protecting groups were removed by reduction with sodium in liquid ammonia,[16] and oxidative cyclization was mediated by ferricyanide.[17] The product was purified by gel filtration with Sephadex G-15 (aqueous acetic acid)[18] and chromatography on Sephadex LH-20 in the solvent system butanol–acetic acid–water–pyridine (15:3:3:10 v/v).

Bioassay Methods. Agonistic and antagonistic properties of this analogue were estimated by methods described previously.[1] These included intravenous antidiuretic assays in rats under ethanol anesthesia, vasopressor assays in phenoxybenzamine-treated rats under urethane anesthesia, and in vitro assays on the rat uterus suspended in a magnesium-free solution.

Agonistic activities are expressed in units per milligram. Antagonistic activities are expressed by pA_2 values following Schild.[19] The pA_2 represents the negative logarithm to the base 10 of the average molar concentration (M) of an antagonist which will reduce the specific biological response to 2*x* units of an agonist to the level of response to *x* units of the agonist.

Results and Discussion

Some pharmacological properties of d(CH_2)$_5$VDAVP are shown in Table I. Previously reported data for those properties of dPVDAVP and dVDAVP are included for comparison.

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Table I. Pharmacological Properties of $d(CH_2)_5$VDAVP, dPVDAVP, and dVDAVP

Peptide	Antidiuretic act., U/mg	Antivasopressor pA_2[a]	Antioxytocic pA_2[a]
$d(CH_2)_5$-VDAVP	0.10 ± 0.02	7.68 ± 0.05 (11)	6.62 ± 0.07 (7)
dPVDAVP[b]	123 ± 22	7.82 ± 0.05	7.23 ± 0.04
dVDAVP	1230 ± 170[c]	7.03 ± 0.11[b]	Agonist[c]

[a] pA_2 values were obtained as described in Manning et al.[1] by the method of Schild[19] by calculating a pA_2 for each assay group; the figures presented are the means ± SE of these. The numbers in parentheses indicate the number of assays. [b] From Manning et al.[1] [c] From Manning et al.[2]

The replacement of the β,β-dimethyl group of dPVDAVP by β,β-cyclopentamethylene has given an analogue, $d(CH_2)_5$VDAVP, with significantly reduced antioxytocic activity. The antivasopressor potency of $d(CH_2)_5$VDAVP may be slightly less than that of dPVDAVP, but this difference is not statistically significant. These vasopressin analogues thus demonstrate different behavior from that of (1-deaminopenicillamine)oxytocin (dPOT) and its cyclopentamethylene analogue [$d(CH_2)_5$OT]. Compared to dPOT, $d(CH_2)_5$OT was reported to have enhanced antioxytocic activity and greatly diminished antivasopressor activity.[7]

The analogue $d(CH_2)_5$VDAVP was found to be an extremely weak antidiuretic agent, its activity of 0.1 unit/mg is some three orders of magnitude less than that of dPVDAVP. There was no evidence, however, that $d(CH_2)_5$VDAVP could antagonize the antidiuretic response to subsequent injections of arginine-vasopressin (AVP). Although not leading to an anti-antidiuretic agent, the further substitution on the β carbon at position 1 has generated from dVDAVP an analogue in which antidiuretic activity has been virtually eliminated. The enhanced antivasopressor potency of dPVDAVP compared to that of dVDAVP has been retained in $d(CH_2)_5$VDAVP. dPVDAVP and $d(CH_2)_5$VDAVP exhibit nearly identical pA_2 values in this regard. Thus the new analogue is dramatically specific pharmacologically as an antagonist of the vasopressor response to AVP. It could thus be a useful pharmacological tool for investigating the possible role(s) of AVP in cardiovascular regulation.

Experimental Section

Trifluoroacetic acid (TFA) was distilled from P_2O_5. *N*-Methylmorpholine (NMM) was distilled from ninhydrin. Dimethylformamide (DMF) was distilled under reduced pressure after having stood over a molecular sieve. *S*-Benzyl-β-mercapto-β,β-cyclopentamethylenepropionic acid was prepared according to the literature method.[7] Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. The analytical results for the elements indicated by their symbols were ±0.4% of theoretical values. For amino acid analysis,[20] samples (~0.5 mg) were hydrolyzed with constant boiling HCl (400 μL) containing phenol (20 μL) in evacuated and sealed ampules for 18 h at 110 °C. The analyses were performed with a Beckman Automatic Amino Acid Analyzer Model 121. Ratios were referred to Gly = 1.00. Optical rotations were measured with a Bellingham Stanley Ltd. Model A polarimeter, type P1. R_f values are for thin-layer chromatography (TLC) on silica gel (Brinkman silplate, 0.25 mm) in the following solvent systems: A, butan-1-ol–acetic acid–water (4:1:5 v/v, upper phase); B, butan-1-ol–water (3.5% in acetic acid, 1.5% in pyridine) (1:1 v/v, upper phase); C, butan-1-ol–acetic acid–water–pyridine (15:3:3:10 v/v). The chloroplatinate reagent, chlorine–potassium iodide–tolidine, and ninhydrin were used for detection.

β-(*S*-Benzylmercapto)-β,β-cyclopentamethylenepropionyl-Tyr(Bzl)-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2. Boc-Tyr(Bzl)-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2[13] (278 mg, 0.2 mmol) was dissolved in cold TFA (2 mL) and left to stand at room temperature for 30 min with occasional vortex mixing. Cold ether (20 mL) was added and the precipitated material centrifuged and washed with ether (three times, aliquot 20 mL) by successive centrifugation and decantation, the precipitate being well suspended in each wash by vortex mixing. The product was dried in vacuo over sodium hydroxide pellets. This material was dissolved in DMF (1 mL) and NMM (40 μL) added gradually to give a solution of pH ~7 to moist pH paper. A solution of β-(*S*-benzylmercapto)-β,β-cyclopentamethylenepropionic acid[7] (132 mg, 0.5 mmol) and HOBt·H_2O (115 mg, 0.75 mmol) in DMF (~1 mL) was cooled in ice and treated with a solution of DCCI (50% w/v) in dichloromethane (206 μL, 0.5 mmol). The reaction mixture (volume 1.7 mL) was left to stand at room temperature for 1 h with occasional mixing. The precipitated dicyclohexylurea was centrifuged, and supernatant (0.85 mL, 0.25 mmol of acylating agent) was added to the neutralized solution of the octapeptide derivative.[15] The mixture was set aside overnight, during which time it formed a gel. The atmosphere above the reaction mixture maintained a slightly alkaline reaction to moist pH paper during this time.[21] The consumption of the ninhydrin-positive material [R_f (A) 0.58] with the formation of a product [R_f (A) 0.65] giving no color with the reagent was demonstrated by TLC (both materials detected by the chloroplatinate reagent and by chlorine–potassium iodide–tolidine). Ethanol (85%, 15 mL) was added with vigorous mixing. The precipitated material was washed with ethanol (95%) and ether (three times with each solvent, aliquot 10 mL) by successive centrifugation and decantation; the precipitate was well suspended in each wash. A solution of the crude product in a minimum quantity of hot DMF was diluted tenfold with boiling methanol giving a solution which, while attaining room temperature, deposited the acyloctapeptide which was filtered, washed with methanol and ether, and dried in vacuo over P_2O_5: yield 218 mg (71%); mp 228–229 °C; R_f (A) 0.65, R_f (B) 0.70, R_f (C) 0.91; $[\alpha]^{22}_D$ -45° (*c* 1, DMF). Anal. ($C_{79}H_{99}N_{13}O_{13}S_3$) C, H, N. Amino acid analysis[20] gave Tyr, 0.89; Phe, 1.04; Val, 1.09; Asp, 1.09; Cys(Bzl), 1.06; Pro, 1.13; Arg, 1.08; Gly, 1.00; NH_3, 2.32.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),4-valine,8-D-arginine]vasopressin. A solution of the foregoing acyloctapeptide (100 mg) in sodium-dried, redistilled ammonia (~300 mL) was treated at the boiling point, with stirring, with sodium[16] from a stick of the metal contained in a small-bore glass tube,[17] until a light blue color persisted in the solution for 15–20 s (~0.01 mL of sodium consumed). The color was discharged by the dropwise addition of dry glacial acetic acid. The solution was evaporated and the residue taken up, with stirring, in aqueous acetic acid (0.2%, 600 mL). Following the addition of aqueous ammonia (2 M) to give a solution of pH ~7 an excess of potassium ferricyanide solution (0.01 M, 13.5 mL) was added gradually. The yellow solution was stirred for a further 10 min; the pH was adjusted to ~5 with glacial acetic acid and for 5 min with anion-exchange resin (Bio-Rad AG-3, chloride form, 10 g damp weight). The suspension was filtered through a bed of the resin (~80 g damp weight). The bed was washed with aqueous acetic acid (0.2%, 200 mL) and the combined filtrate and washings were lyophilized. The resulting powder was desalted on Sephadex G-15 (column 110 × 2.7 cm) eluting with aqueous acetic acid (50%) with a flow rate ~4 mL h^{-1}.[2,18] The eluate was fractionated and monitored for absorbance (280 nm) and by TLC. The major peak of the peptide material was isolated by lyophilization (56 mg) and further subjected to gel filtration on Sephadex G-15 (column 100 × 1.5 cm), eluting with aqueous acetic acid (0.2 M) with a flow rate ~4 mL h^{-1}.[2,18] The fractions of the eluate containing the peptide were cloudy and contained traces of component(s) slightly less mobile on TLC than the bulk product. The material isolated from these fractions by lyophilization (55 mg) was chromatographed on Sephadex LH-20 (column 100 × 1.5 cm) in solvent system C (flow rate 6.5 mL h^{-1}, fraction 2.5 mL). The fractions containing the product (TLC) were pooled and evaporated to near dryness (bath temperature 30 °C), and the evaporation was twice repeated following the addition of aqueous acetic acid (50%, aliquot 5 mL). The analogue was then isolated by lyophilization (twice) from aqueous acetic acid (1%, 10 mL): yield 41 mg; homogeneous (load 50 μg) to TLC, R_f (A) 0.16 and R_f (C) 0.70, and to electrophoresis in aqueous acetic

acid (30%, 450 V, 4 h); $[\alpha]^{22}_D$ -42° (c 0.5, 1 M acetic acid). Anal. ($C_{51}H_{73}N_{13}O_{11}S_2 \cdot 3CH_2COOH \cdot 5H_2O$) C, H, N. Amino acid analysis gave Tyr, 0.95; Phe, 0.98; Val, 0.98; Asp, 0.98; $^1/_2$Cys, 0.40; Pro, 1.01; Arg, 0.86; Gly, 1.00; NH_3, 1.83. Analysis following performic acid oxidation[22] gave a Cys(O_3H):Gly ratio of 1.03:1.00.

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Book Reviews

Annual Review of Pharmacology and Toxicology. Volume 17. Edited by H. W. Elliott, R. George, and R. Okun. Annual Reviews, Palo Alto, Calif. 1977. 750 pp. 15 × 22 cm. $17.00.

This latest volume of "Annual Reviews" is dedicated to Professor Henry W. Elliott who helped to establish this series and guided the "Annual Reviews" to become an important reference in pharmacology.

The prefactory chapter of this volume is an autobiographical sketch of Karl H. Beyer, Jr., who has retired from a highly successful career in industry after making major contributions in renal pharmacology and now is enjoying a new career in academia.

There are 37 review articles presented in this volume covering a wide range of pharmacological and toxicological subjects. Cardiovascular pharmacology is surveyed in four articles including the effects of calcium on myocardial and smooth muscle (A. Fleckenstein), cardiovascular drug interactions (D. Craig Brater and H. F. Morrelli), combination drug therapy of hypertension (C. T. Dollery), and the effect of hypolipidemic drugs on plasma lipoproteins. Renal pharmacology is discussed in two reviews including a discussion on the renal lithiasis (T. H. Steele) and a review on proximal tubular reabsorption (H. R. Jacobson and D. W. Seldin).

A number of articles deal with neuropharmacology, psychopharmacology, and drug–receptor interactions. J. W. Maas discusses the effects of various psychopharmacological agents on biogenic amine metabolism in the central nervous system, whereas J. L. Diaz discusses various aspects of the use of psychotropic plants by the Mexican Indians. Peptide neurotransmitters such as Substance **P**, proctolin, and enkephalin are discussed by M. Otsuka and T. Takahashi, whereas the effects of prostaglandins on autonomic transmission are discussed by P. Hedqvist. In addition, J. Schwartz reviews putative histaminergic mechanisms in the brain and O. Hornykiewicz examines dopamine and dopamine antagonists. The dynamic regulation of cholinergic function is discussed by D. L. Cheney and E. Costa in an article on acetylcholine turnover in rat brain. The interaction of catecholamine with its receptor and the activation of adenylyl cyclase are reviewed by B. B. Wolf, T. K. Harden, and P. B. Molinoff, whereas B. Weiss and W. N. Hait examine the role of cyclic nucleotides and the possible therapeutic use of inhibitors of cyclic nucleotide phosphodiesterases. In addition, receptor activation and the evolution of receptor proteins using aquatic invertebrates as model systems for study are summarized by H. M. Lenhoff and W. Heagy.

Review articles concerning the pharmacology of central homeostatic control mechanisms include the control of feeding (B. G. Hoebel) and the control of temperature and thermoregulation (B. Cox and P. Lomax). The pharmacology of local anesthetics and regional pain relief are discussed by J. Adriani and M. Naraghi. The pharmacology of experimental myopathies and its application to the development of models for progressive muscular dystrophies are reviewed by M. B. Laskowski and W. D. Dettbarn.

Cancer and cancer-related articles include a review on the chemistry of selected antineoplastic agents from plants (M. E. Wall and M. C. Wani), the application of cell kinetics to clinical cancer therapy (R. B. Livingston and J. S. Hart), and the immunological aspects on cancer chemotherapy (C. M. Haskell). In addition, the thymus and the role of thymic hormones in control of the immune system are reviewed by J. F. Bach.

Several articles concerning endocrine pharmacology include a discussion on the mechanisms of hormone secretion (J. M. Trifaro) and the clinical pharmacology of systemic corticosteroids (J. C. Melby).

Age and its effects on pharmacological agents is the subject of two reviews including pediatric clinical pharmacology (A. K. Done, S. N. Cohen, and L. Strebel) and the problems of aging on pharmacokinetics (D. P. Ritchey and A. D. Bender).

Miscellaneous, pharmacologically related articles include the pharmacology of laxatives (H. J. Binder), the pharmacology of magnesium (S. G. Massry), the therapeutic use of enzymes (J. S. Holcenberg and J. Roberts), and the pharmacological effects of hymenoptera venoms (R. I. Levy).

Although it is somewhat difficult to distinguish articles concerning pure pharmacology from those concerned only with