

- 12, 951 (1964).
 (15) J. F. Gerster, J. W. Jones, and R. K. Robins, *J. Org. Chem.*, 28, 945 (1963).
 (16) H. J. Schaeffer and D. Vogel, *J. Med. Chem.*, 8, 507 (1965).

- (17) H. J. Schaeffer, S. Gurwara, R. Vince, and S. Bittner, *ibid.*, 14, 367 (1971).
 (18) H. Brederbeck, *Chem. Ber.*, 80, 401 (1947).
 (19) N. O. Kaplan, *Methods Enzymol.*, 2, 473 (1955).

Analogs of *S*-Adenosylhomocysteine as Potential Inhibitors of Biological Transmethylation. Specificity of the *S*-Adenosylhomocysteine Binding Site[†]

James K. Coward* and Edwin P. Slisz

Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510. Received November 27, 1972

Several structural analogs of *S*-adenosylhomocysteine have been synthesized and their interaction with catechol *O*-methyltransferase (COMT) has been investigated. Kinetic studies on the inhibition of COMT by these compounds have resulted in a delineation of binding forces involved in the inhibition of transmethylation by *S*-adenosylhomocysteine. These data demonstrate a strict specificity in the interaction of *S*-adenosylhomocysteine with COMT. This specificity may be critical in the regulation of biological transmethylation by *S*-adenosylhomocysteine.

The general utilization of *S*-adenosylmethionine (SAM)[‡] as a methyl donor in numerous biological reactions has been known for many years.¹ Inhibition by the demethylated product, *S*-adenosylhomocysteine (SAH), seems to be a general feature of many of these enzyme-catalyzed transmethylation.² The product inhibition of these methylases presumably is regulated by further breakdown of SAH. It has recently been shown that there is present in the rat brain a hydrolase (*S*-adenosylhomocysteinase) which can degrade SAH and apparently exert a regulatory role in the activity of phenethanolamine *N*-methyltransferase (PNMT).³ Most studies of the interaction between small molecules and various methylases have been concerned only with the acceptor portion, *e.g.*, catecholamine analogs as inhibitors of PNMT⁴ and catechol *O*-methyltransferase (COMT, E.C. 2.1.1.6).^{5,6} More recently, the use of substituted adenosines (cytokinins) as inhibitors of tRNA methylases has been reported.⁷ In order to study the nature of the intermolecular forces involved in the binding of SAH to various methylases, we have continued our earlier studies⁸ and synthesized a series of compounds with modifications in the nucleoside portion of the SAH molecule (Chart I). Use of these compounds as probes of the COMT active site permits elucidation of the

contribution made by specific moieties of SAH in regulation of methylase activity.

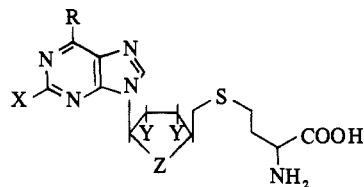
Experimental Section

All melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements or functions were within $\pm 0.4\%$ of the theoretical values. IR spectra were run on a Perkin-Elmer Model 21 spectrophotometer and uv spectra on a Cary Model 15 spectrophotometer. Tlc were run on Eastman chromatograms No. 6060 (silica gel with fluorescent indicator) or No. 6065 (cellulose with fluorescent indicator). Spots were detected by visual examination under uv light and/or with ninhydrin, or Ptl₄ spray reagents, for compounds containing amino or thioether moieties, respectively.

COMT was isolated from rat liver and purified as described by Nikodejevic, *et al.*,⁶ with minor modifications. Enzyme assays were carried out as described previously;^{8,9} substrates and other materials required for the assays were obtained as indicated in the references cited. *S*-Inosylhomocysteine (4) was prepared as described by Zappia, *et al.*¹⁰ Aristeromycin was a gift from Dr. T. Kishi of Takeda Chemical Industries, Ltd. 2-Fluoroadenosine was supplied by Drug Research and Development, Chemotherapy, National Cancer Institute. 9-[*S*-(4-(2-Amino)butyric acid)-*S'*-thiopentyl]adenine (5) was prepared as previously described.⁸ *S*-Adenosylhomocysteine sulfoxide (SAHO) was prepared by the method of Duerre, *et al.*¹¹

cis-1'-(6-Amino-9-puriny)-3'-*S*-cyclopentylmethylhomocysteine (1). *cis*-3-(6-Amino-9-puriny)cyclopentylcarbinol¹² was prepared by modified literature procedures. This alcohol (1.63 g, 7.02 mmol) was dissolved in 25 ml of dry pyridine, freshly distilled from barium oxide, and the resulting solution cooled to *ca.* 0° in an ice-salt bath. Freshly recrystallized *p*-toluenesulfonyl chloride (1.49 g, 7.89 mmol) was added to the cooled solution in one portion and stirring continued for 5 min. The reaction solution was removed from the ice bath and allowed to stand overnight at ambient temperature. Evaporation of the pyridine *in vacuo* was followed by partitioning the residue between CHCl₃ and 3 *N* H₂SO₄ at 4°. The chloroform layer was then washed again with 3 *N* H₂SO₄, H₂O, and finally four times with a saturated solution of NaHCO₃. The dried chloroform layer was then concentrated *in vacuo* to give 1.8 g (67%) of an oily residue, sufficiently pure for further transformations. This oily tosylate was not soluble in liquid ammonia; therefore it was dissolved in 12 ml of bis(2-ethoxyethyl) ether which had been dried over calcium hydride and distilled from LiAlH₄. A solution of homocysteine in liquid NH₃ was generated by adding Na in small pieces (*ca.* 300 mg) to 815 mg (3.6 mmol) of *S*-benzylhomocysteine¹³ until a blue color persisted for 15 min. The tosylate solution was added in one portion and the resulting mixture stirred vigorously at -70° for 10 min and allowed to warm slowly to ambient temperature overnight. After evaporation of residual NH₃, the gummy residue was dissolved in 25 ml of H₂O and the pH adjusted to 4 with 1 *N* H₂SO₄. Extraction with CHCl₃, followed by lyophilization of the aqueous layer, gave a crude product which could be purified by chromatography on Dowex 1 as described previously for related *S*-alkylhomocysteines.⁸ The purified product was a

Chart I. Compounds Synthesized to Probe the SAH Binding Site of Catechol *O*-Methyltransferase

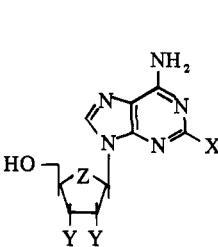
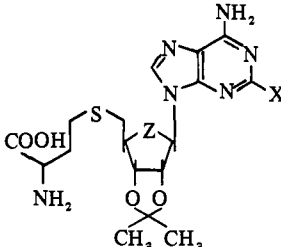
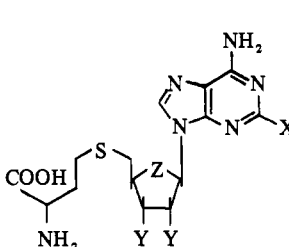


Compd	X	Y	Z	R
SAH	H	OH	O	NH ₂
1	H	H	CH ₂	NH ₂
2	H	OH	CH ₂	NH ₂
3	F	OH	O	NH ₂
4	H	OH	O	OH
5	H	-(CH ₂) ₅ -		NH ₂

[†]This research was supported by funds from the Public Health Service, Grant No. MH-18,038 and CA-10,748.

[‡]Abbreviations used: SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; PNMT, phenethanolamine *N*-methyltransferase; COMT, catechol *O*-methyltransferase; SAHO, *S*-adenosylhomocysteine sulfoxide.

Table I. R_f Values for SAH Analogs and Synthetic Intermediates^a

			
X = H; Y = OH; Z = O	0.69	0.78	0.53 (SAH)
X = F; Y = OH; Z = O	0.73	0.80	0.58 (3)
X = H; Y = OH; Z = CH ₂	0.74	0.80	0.57 (2)
X = H; Y = H; Z = CH ₂	n.d. ^b		0.72 (1)
X = H; Y = H; Z omitted	n.d. ^b		0.76 ^c (5)

^aAll chromatograms were run on cellulose in 1-butanol-acetic acid-water (10:3:9). ^bn.d. = not determined. ^cData of ref 8:

white crystalline material, mp 180–190° with preliminary softening. The product has λ_{\max} 261 nm (ϵ 1.09 \times 10⁴) and was homogeneous by tlc on cellulose (BuOH-HOAc-H₂O, 10:3:9), R_f 0.72. Anal. (C₁₅H₂₂N₆O₂S·2H₂O) C, N; H: calcd, 6.78; found, 6.30.

cis-1'-(6-Amino-9-purinyloxy)-4' β -S-(2 α ,3 α -dihydroxy)cyclopentylmethylhomocysteine (2). The carbocyclic analog of adenosine, aristeromycin^{14,15} (100 mg, 0.38 mmol), was converted to the 2',3'-isopropylidene derivative by standard procedures.¹⁶ The product obtained on work-up of the reaction mixture was homogeneous by tlc on cellulose (*i*-PrOH-NH₄OH-H₂O, 7:1:2), R_f 0.89, and was used directly for the next step. Tosylation of the 5'-hydroxyl group, followed by treatment of the tosylate in liquid NH₃ with homocysteine as described for 1, afforded the crude 2',3'-isopropylidene derivative of 2. Hydrolysis of this material with 1 *N* H₂SO₄ gave crude 2 as a fine white powder. Analytical tlc on cellulose (BuOH-HOAc-H₂O, 10:3:9) demonstrated the presence of a major uv-absorbing, ninhydrin-positive spot (R_f 0.57) together with a smaller spot corresponding to homocystine; λ_{\max} 261 nm. This material was sufficiently pure to use for COMT inhibition studies in view of the fact that separate experiments showed that homocystine had no inhibitory effect on the COMT reaction. The small amount of 2 available, coupled with the high concentrations required for enzyme studies, precluded further purification of the product.

S-(2-Fluoroadenyl)homocysteine (3). 2-Fluoroadenosine¹⁷ (500 mg, 1.75 mmol) was converted to the 2',3'-isopropylidene derivative by standard procedures.¹⁶ The product obtained on work-up of the reaction mixture was homogeneous by tlc on cellulose (*i*-PrOH-NH₄OH-H₂O, 7:1:2), R_f 0.72, and was used directly for the next step. In a manner exactly analogous to that described for 2, the isopropylidene was converted to the 5'-tosylate. The tosylate was dissolved in liquid NH₃ and then treated with homocysteine in liquid NH₃ to give the crude blocked product. Hydrolysis of the 2',3'-isopropylidene group with 1 *N* H₂SO₄ followed by chromatography on Dowex-1 separated all ninhydrin-positive impurities but failed to remove a uv-absorbing material which remained close to the origin on cellulose tlc (BuOH-HOAc-H₂O, 10:3:9). Purification by preparative tlc on cellulose in the same solvent system afforded the desired compound as a fluffy white powder which decomposed over a broad range from 50 to 180°. Anal. (C₁₄H₁₇FN₆O₂S·2H₂O) C, N; H: calcd, 5.27; found, 4.60.

Results and Discussion

The analogs of SAH synthesized for this study were characterized by several methods, including elemental analyses and spectral and chromatographic properties (Table I). Initial inhibition studies were carried out using ¹⁴CH₃-SAM as the radioactive label.⁸ The results of these studies are given in Table II. From these data, it is apparent that neither 1 nor 2 has the potent inhibitory action of SAH against COMT, whereas the 2-fluoro analog 3 is an effective inhibitor, albeit not as good as SAH. Kinetic studies were carried out using 7-³H-epinephrine as the radioactive label.⁹ A graphical representation of these kinetic data is shown in Figure 1. The high concentrations of 1 and 2

Table II. Inhibition of COMT by Analogs of SAH^a

Compd	Concn, mM ^b	% inhibition
SAH	0.91	82.5
1	1.46	0
	3.44	10.2
2	1.0	0
3	1.3	70.0
4	0.85	9.2
Homocystine	1.1	0

^aAssays were carried out as described previously⁸ except that [SAM] = 1.05 mM. ^bConcentrations of SAH and 1–4 in stock solutions were determined by ultraviolet spectroscopy, using molar extinction coefficients for the corresponding nucleosides.

Table III. Binding of SAH and Analogs to COMT. Kinetic and Thermodynamic Constants

Compd	K_i , mM	$-\Delta F$, kcal/mol
SAH ^a	0.05	6.10
3	0.9	4.31
4 ^b	10	2.83
1	13.6	2.65
5 ^{b,c}	15	2.50
SAHO ^{b,d}	1	4.25

^aCalculated from data of ref 9. ^bCalculated from per cent inhibition data. ^cCalculated from data of ref 8. ^dCalculated from data of ref 2.

which were required to demonstrate significant inhibition of COMT (Table II) precluded a detailed kinetic study of the inhibition of COMT by 2. However, based on the data of Table II, it is reasonable to state that the 2',3'-hydroxyl groups of 2 do not lead to a large change in K_i for 2 vs. K_i for 1. The data of Figure 1 lead to the K_i values and negative free energies of binding ($-\Delta F$) listed in Table III. Similar data calculated from previous studies are also included for comparison.

There are many pitfalls associated with the use of analog compounds to probe the nature of enzyme-substrate and enzyme-inhibitor interactions. Of critical importance is the establishment of similar binding sites for both analog and natural substrate or inhibitor. In the present study, we have made systematic changes in the basic SAH molecule. It is not likely that changing a ribose moiety to a dihydroxycyclopentyl group will result in binding to grossly different sites on the enzyme. In fact, data in the literature^{18–20} indicate that cyclopentyl analogs serve as replacements for adenosine derivatives in a variety of enzyme reactions. In the present work, cyclopentyl analogs of SAH were designed to resist enzyme-catalyzed degradation reactions,

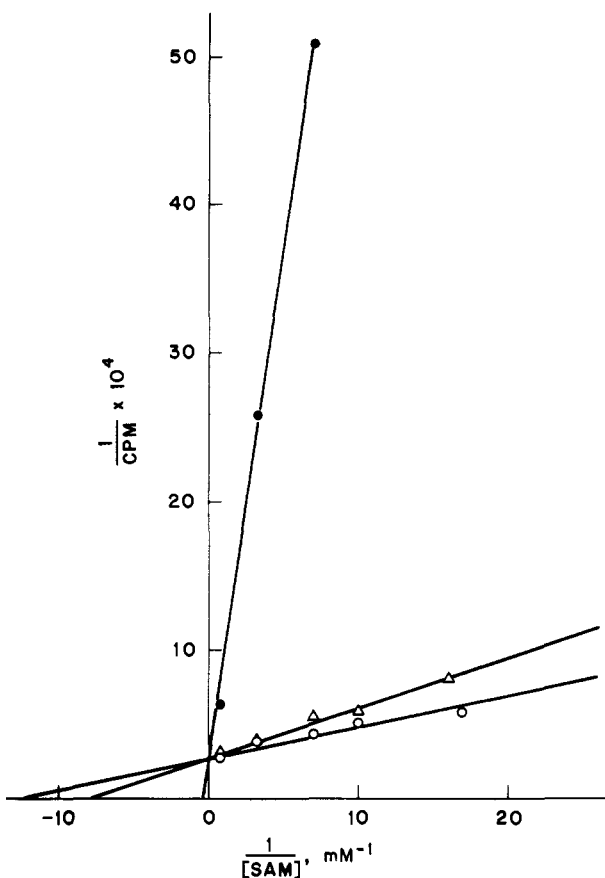


Figure 1. Inhibition of COMT-catalyzed transmethylation. Assay conditions are as described in the text, in the presence of no inhibitor (O), 3.82 mM 1 (Δ), and 0.87 mM 3 (\bullet).

such as that discussed by Chung and Law²¹ which metabolize SAH to adenine and ribosylhomocysteine. This stability to further metabolic breakdown is of considerable importance for future pharmacological studies *in vivo*. The data of Figure 1 show that 1 is inhibiting the COMT reaction in a competitive manner with respect to SAM. This is in agreement with the results of product inhibition studies carried out recently in this laboratory.⁹ This supports, but does not prove, the identity of the binding sites for SAH and 1 (and, presumably, 2). The large difference in K_i values suggests that in making the structural changes from SAH to 1 and 2, some of the interactions critical to formation of the E-I complex have been grossly disturbed. The differences in negative free energy of binding for SAH and 1 is *ca.* 3.5 kcal/mol (Table III). It should be noted that removal of the steric constraints of 1 to give 5 has little effect on the binding energy. Assuming that the 2',3'-hydroxyl groups contribute very little to the binding interaction (*vide infra*), most of this change in free energy of binding can be associated with replacing the furanose oxygen of ribose with a methylene group. The inhibition of COMT by 3 indicates that it is possible to make small changes in the adenine ring portion of SAH without losing the potent activity of SAH. Similarly, the activity of SAHO² shows that it is possible to alter the sulfur atom of SAH without a large loss of activity. However, it is not possible to make changes which affect the hybridization of the ring atoms, *i.e.*, SAH \rightarrow 4. In this case, the N_1 of the adenine ring is converted from an imino nitrogen to an amide nitrogen,²² and the resultant electronic and steric changes in the purine ring obviously have a deleterious effect on formation of the E-I complex. The 2-fluoro analog 3 may

be susceptible to the degradative hydrolytic enzymes discussed by Chung and Law²¹ and thus is not the optimal candidate for *in vivo* experiments. However, it acts as a competitive inhibitor with respect to SAM and therefore should be a useful ¹⁹F nmr probe²³ of the active site of COMT and other methylases.

The difference in binding energy between SAH and 1 is quite large but is not without precedent. Bennett and co-workers¹⁸ observed large differences in K_m values between adenosine and the cyclopentyl analog for adenosine deaminase and adenosine kinase. If one makes the simplifying assumption that K_m approximates a binding constant, a difference of *ca.* 2.5 kcal/mol is obtained for binding of adenosine *vs.* the cyclopentyl analog. Similarly, Santi, *et al.*,²⁰ found a difference of *ca.* 1.0 kcal/mol from K_i values of adenosine *vs.* the cyclopentyl analog acting as inhibitors of ATP-PP_i exchange, catalyzed by phenylalanine tRNA synthetase. In contrast, Kerwar, *et al.*,¹⁹ found that 5,6-dimethylbenzimidazolylcobamide-5'-deoxyadenosine, and the carbocyclic analog, had very similar K_m values in the dioldehydrase system.

While this work was in progress, two publications appeared^{24,25} in which adenosine, guanosine, and uridine derivatives were synthesized as potential inhibitors of methyltransferases. Unfortunately, no data were presented concerning the biological activity of these compounds. It is apparent from the present work and from previous work in this laboratory⁸ that the sugar and amino acid moieties of SAH are required in order to obtain potent inhibition of COMT. Retention of inhibitory activity similar to SAH is observed only when very slight modifications are made in the purine ring, *e.g.*, 3. The low inhibitory activity of most SAH analogs prepared to date, coupled with the high degree of specificity of several methylases for the substrate, SAM,¹⁰ indicates that methylases have very specific binding sites. Only a slight variation in the structure of SAM or SAH eliminates most substrate or inhibitor activity. This may be another manifestation of the critical role played by SAM and SAH in the regulation of numerous cellular reactions.²⁶

References

- (1) S. K. Shapiro and F. Schlenk, Ed., "Transmethylation and Methionine Biosynthesis," The University of Chicago Press, Chicago, Ill., 1965.
- (2) J. K. Coward, M. D'Urso-Scott, and W. D. Sweet, *Biochem. Pharmacol.*, **21**, 1200 (1972).
- (3) T. Deguchi and J. Barchas, *J. Biol. Chem.*, **246**, 3175 (1971).
- (4) R. W. Fuller, J. Mills, and M. M. Marsh, *J. Med. Chem.*, **14**, 322 (1971).
- (5) A. D'Iorio and C. Mavrides, *Can. J. Biochem. Physiol.*, **41**, 1779 (1963).
- (6) B. Nikodejevic, S. Senoh, J. W. Daly, and C. R. Creveling, *J. Pharmacol. Exp. Ther.*, **174**, 83 (1970).
- (7) E. Wainfain and B. Landsberg, *FEBS Lett.*, **19**, 144 (1971).
- (8) J. K. Coward and W. D. Sweet, *J. Med. Chem.*, **15**, 381 (1972).
- (9) J. K. Coward, E. P. Slisz, and F. Y.-H. Wu, *Biochemistry*, in press.
- (10) V. Zappia, C. R. Zydek-Cwick, and F. Schlenk, *J. Biol. Chem.*, **244**, 4499 (1969).
- (11) J. A. Duerre, L. Salisbury, and C. H. Miller, *Anal. Biochem.*, **35**, 505 (1970).
- (12) H. J. Schaeffer, D. D. Godse, and G. Liu, *J. Pharm. Sci.*, **53**, 1510 (1964).
- (13) M. D. Armstrong and G. B. Brown, *Biochem. Prep.*, **5**, 91 (1957).
- (14) T. Kishi, T. Muroi, T. Kusaka, M. Nishikawa, K. Kamiya, and K. Mizuno, *Chem. Commun.*, 852 (1967).
- (15) Y. F. Shealy and J. D. Clayton, *J. Amer. Chem. Soc.*, **91**, 3075 (1969).
- (16) A. Hampton, *ibid.*, **83**, 3640 (1961).
- (17) J. A. Montgomery and K. Hewson, *ibid.*, **82**, 463 (1960).

- (18) L. L. Bennett, Jr., P. W. Allan, and D. C. Hill, *Mol. Pharmacol.*, **4**, 208 (1968).
 (19) S. S. Kerwar, T. A. Smith, and R. H. Abeles, *J. Biol. Chem.*, **245**, 1169 (1970).
 (20) D. V. Santi, P. V. Danenberg, and K. A. Montgomery, *Biochemistry*, **10**, 4821 (1971).
 (21) A. Chung and J. Law, *ibid.*, **3**, 1989 (1964).
 (22) J. H. Lister in "Fused Pyrimidines, Part II, Purines," D. J. Brown, Ed., Wiley-Interscience, New York, N. Y., 1971, p 8.
 (23) S. H. Smallcombe, K. L. Gammon, and J. H. Richards, *J. Amer. Chem. Soc.*, **94**, 4585 (1972), and references cited therein.
 (24) J. Hildesheim, R. Hildesheim, and E. Lederer, *Biochimie*, **53**, 1067 (1971).
 (25) J. Hildesheim, R. Hildesheim, and E. Lederer, *ibid.*, **54**, 431 (1972).
 (26) J. B. Lombardini and P. Talalay, *Advan. Enzyme Regul.*, **9**, 349 (1971).

Solid-Phase Synthesis and Some Pharmacological Properties of 4-Threonine Analogs of Vasopressins and Vasotocin and of Arginine-vasopressin and Arginine-vasotocin[†]

Maurice Manning,* Esther J. Coy,

Department of Biochemistry, Medical College of Ohio, Toledo, Ohio 43614

Wilbur H. Sawyer, and Margot Acosta

Department of Pharmacology, College of Physicians and Surgeons of Columbia University, New York, New York 10032.

Received October 24, 1972

[4-Threonine]arginine-vasopressin I, [4-threonine]lysine-vasopressin II, [4-threonine]arginine-vasotocin III, arginine-vasopressin IV, and arginine-vasotocin V were synthesized using the solid-phase method. The deblocked and reoxidized peptides were purified by sequential gel filtration on Sephadex G-15 in 50% AcOH and 0.2 *N* AcOH. By comparison with the parent 4-glutamine-containing peptide in each case I-III exhibited: (a) increases in rat uterus activity of 34, 28, and 58% and in fowl vasodepressor activity of 200, 400, and 69%, respectively; (b) decreases in rat vasopressor activity of 71, 80, and 21%. With respect to the antidiuretic activity, I and II gave decreases of 28 and 45% whereas III had an increase of 21%. The substitution of threonine for glutamine therefore has brought about a selective increase of the antidiuretic to pressor ratio in all three analogs. The potencies of IV and V were in general agreement with those in the literature and were used for comparisons with those of I and III.

Substitution of threonine for glutamine in position 4 of the neutral neurohypophysial principles, oxytocin and mesotocin, gave rise to analogs possessing quite remarkable properties. On the one hand, oxytocin-like activities were markedly enhanced and, on the other, vasopressin-like activities were decreased.^{1b-3} These findings immediately raised the question: how would a similar threonine/glutamine interchange affect the characteristic activities of the basic neurohypophysial peptides, arginine-vasopressin, lysine-vasopressin, and arginine-vasotocin? This study was therefore carried out (a) to provide an immediate answer to this question, (b) to determine whether or not the effects produced might fit into a predictable pattern, and (c) to give some further insight into the role of the amino acid in position 4 in determining the biological characteristics of the basic neurohypophysial peptides. In addition to reporting on the synthesis and pharmacological properties of these 4-threonine analogs, we present here also independent syntheses and pharmacological properties of both arginine-vasopressin and arginine-vasotocin. Both of these compounds had been synthesized by a number of investigators using a variety of classical methods of peptide synthesis.⁴ However, no reports on the synthesis of either compound by the solid-phase method^{5,6} had appeared when these syntheses were first undertaken. Following completion of

the present synthesis of arginine-vasopressin, a solid-phase synthesis of a preparation possessing very high antidiuretic and pressor activities was reported.⁷ However, our approach is sufficiently distinct to justify its inclusion in the present report. It should be noted also that syntheses of both arginine-vasopressin and arginine-vasotocin by the solid-phase method have very recently been reported elsewhere,⁸ but no details of either the syntheses or of the characteristic pharmacological properties were given. All of the required protected nonapeptide intermediates were synthesized using the Merrifield method^{5,6} as adapted for the synthesis of oxytocin,⁹ [8-phenylalanine]oxytocin,¹⁰ and [4-threonine]oxytocin² by either manual or automated methods, and the final purified compounds were obtained by previously described methods¹¹⁻¹⁴ as outlined in the Experimental Section. Measurements of the pharmacological potencies were carried out as previously described.¹⁵

Results and Discussion

The data presented in Table I show that substitution of threonine for glutamine in the 4 position of all three basic neurohypophysial peptides increased oxytocic and fowl vasodepressor activities. These changes closely parallel in a qualitative but not in a quantitative sense those observed when threonine is substituted for glutamine in oxytocin and mesotocin. Thus, the [4-threonine] analogs of arginine-vasopressin, lysine-vasopressin, and arginine-vasotocin exhibited enhancements in rat uterus activity of 34, 28, and 58%, respectively, whereas the corresponding value for [4-threonine]oxytocin was 80%. All three [4-threonine]-substituted analogs of the basic neurohypophysial peptides demonstrated substantially increased potencies in fowl vasodepressor activity of 200, 400, and 69% when compared with

[†]This work was supported in part by Research Grants from the National Institute of Child Health and Human Development No. 1R01HD06351-01A1, the National Science Foundation No. GB-30598X, the National Institute of Arthritis and Metabolic Diseases No. AM-01940, and by General Research Support Grants to the Medical College of Ohio and to Columbia University from the National Institute of Health. An abstract of part of this work was presented at the American Society of Biological Chemists Meeting, San Francisco, Calif., June 1971; see also ref 1a. All optically active amino acids are of the L variety.