positive to an accuracy of 10 standard deviations, based primarily on the accuracy with which the upper-state population is determined (± 10 percent). The derived gain would result in gain narrowing of the line by ~ 10 kHz, an unobservable amount compared with the kinetic line widths. However, the atmospheric gain should be substantially greater at large zenith angles than in the zenith direction, and a search for macroscopic gain narrowing seems warranted.

In conclusion, natural gain amplification has been observed in the mesophere of Mars, representing to our knowledge the first definite identification of a natural infrared laser.

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1. Plausible candidates have been presented as possible natural lasers, including NH_3 10-μm emission from the Jovian polar regions [T. Kostiuk, M. J. Mumma, J. J. Hillman, D. Buhl, L. W. Brown, J. Faris, D. L. Spears, *Infrared Phys.* **17**, 431 (1977); M. J. Mumma, in *Proceedings of the International Conference on Lasers '78*, V. J. Corcoran, Ed. (STS Press, McLean, Va., 1979), p. 700] and atomic hydrogen emission (Pfund β, near 4.65 μm) from the Becklin-Neugebauer infrared object [H. Smith, H. Larson, U. Fink, *Astrophys. J.* **233**, 132 (1979)]. For a recent review of natural astrophysical masers, see L. E. Snyder, in *Interstellar Molecules*, B. H. Andrew, Ed. (Reidel, Dordrecht, 1980).
2. A similar CO_2 laser–infrared heterodyne spectrometer is described by M. J. Mumma, T. Kostiuk, and D. Buhl [*Opt. Eng.* **17**, 50 (1978)]. The heterodyne spectrometer was placed at the McMath solar telescope at Kitt Peak National Observatory.
3. The instrumental field of view was placed tangent to the planetary limb to minimize possible underfilling of the beam during measurements near the limb.
4. These bright core emissions were first reported by Johnson *et al.* (*5*). See also A. L. Betz, R. A. McLaren, E. C. Sutton, and M. A. Johnson [*Icarus* **30**, 650 (1977)]. These authors derived kinetic temperatures of 150 to 200 K from the core emission line widths, much larger than the present results and substantially larger than the Viking probe data. However, their observations accepted about twice the planetary area ours did, possibly leading to broadening of their observed lines by planetary rotation.
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14. We also calculated the gain for a kinetic temperature of 151 K corresponding to our "high-temperature" line and find positive definite gain with the source function bound even for that case. However, were the temperature to be as high as 170 K, as observed by Johnson *et al.* (*5*), we would find negative gain (absorption). We also calculated the gain based on a mean Viking isothermal temperature of 140 K and found positive gain for that case, as well.
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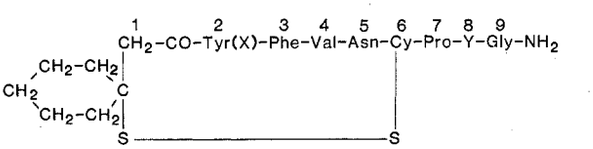
Vasopressin Analogs That Antagonize Antidiuretic Responses by Rats to the Antidiuretic Hormone

Abstract. *Four new synthetic analogs of vasopressin (antidiuretic hormone) can antagonize the antidiuretic response to intravenous vasopressin in anesthetized, water-loaded rats. They also cause a diuresis resembling that of diabetes insipidus when given intraperitoneally to conscious rats. Such antagonists may prove to be useful both pharmacologically and therapeutically.*

The development of useful synthetic antagonists of in vivo antidiuretic responses to arginine vasopressin (the antidiuretic hormone or ADH) has proved to be an elusive goal. Hundreds of analogs of the neurohypophysial peptides oxytocin and vasopressin have been synthesized and pharmacologically evaluated over the past 27 years. A number of these can effectively antagonize vasopressor responses to vasopressin in vivo (*1–3*). Of the few analogs that appeared to antagonize antidiuretic responses to ADH (*4, 5*), none has emerged as a pharmacologically or clinically useful antidiuretic antagonist. We now report four new synthetic analogs of arginine vasopressin that effectively antagonize antidiuretic responses by rats to exogenous or endogenous ADH. These analogs, in order of their increasing potencies in antagonizing the antidiuretic response, are **1**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-*O*-methyltyrosine, 4-valine, 8-D-arginine]vasopressin, abbreviated as $d(CH_2)_5$Tyr(Me)VDAVP; **2**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-*O*-ethyltyrosine, 4-valine, 8-D-arginine]vasopressin, abbreviated as $d(CH_2)_5$Tyr(Et)VDAVP; **3**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-*O*-methyltyrosine, 4-valine]arginine vasopressin, abbreviated as $d(CH_2)_5$Tyr(Me)VAVP; and **4**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-*O*-ethyltyrosine, 4-valine]arginine vasopressin, abbreviated as $d(CH_2)_5$Tyr(Et)VAVP. These analogs have the general structure shown in Fig. 1.

The analogs were designed by modifying one of our previously reported antagonists of vasopressor responses to vasopressin, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 4-valine, 8-D-arginine]vasopressin [$d(CH_2)_5$-VDAVP; X = H, Y = D-Arg] (*3*). Although not an effective antagonist of antidiuretic responses to ADH in vivo, this analog had been shown to be a competitive antagonist of the activation of renal medullary adenylate cyclase by ADH in vitro (*6*). The work of Larsson *et al.* (*5*) pointed to the feasibility of using *O*-alkyltyrosine substitutions in attempts to convert this peptide into an antagonist of the antidiuretic response in vivo. Such substitutions in the highly potent antidiuretic agonist deamino-lysine-vasopressin had resulted in peptides with antagonistic activity, albeit not dose-related, of antidiuretic responses to lysine vasopressin in rats (*5*). Analogs **1** and **2** were thus designed by incorporating *O*-methyl



Analog	X	Y
1	CH_3	D-Arg
2	C_2H_5	D-Arg
3	CH_3	L-Arg
4	C_2H_5	L-Arg

Fig. 1. The structure of analogs **1** to **4**.

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Table 1. Antagonism of antidiuretic responses to ADH by analogs injected intravenously into ethanol-anesthetized rats.

Analog	"Effective dose"* (nmole/kg)
1 $d(CH_2)_5Tyr(Me)V_DAVP$	15.1 ± 3.5
2 $d(CH_2)_5Tyr(Et)V_DAVP$	5.7 ± 1.0
3 $d(CH_2)_5Tyr(Me)VAVP$	3.1 ± 0.4
4 $d(CH_2)_5Tyr(Et)VAVP$	1.9 ± 0.2

*The "effective dose" is the dose of an antagonist that reduces the antidiuretic response to 2x units of ADH injected intravenously 20 minutes after the antagonist to equal the responses to 1x units of ADH injected before the antagonist. This is estimated by finding doses of the antagonist that are above and below the "effective dose" which is then obtained by interpolation on a logarithmic scale. Each figure represents the mean ± standard error of four such assays on four rats. For these analogs, 1 nmole is 1.12 or 1.14 μg.

and *O*-ethyl substituents on the tyrosine residue at position 2 in $d(CH_2)_5V_DAVP$. Modification of these two analogs by replacing D-arginine by L-arginine to give analogs **3** and **4** brought about further enhancement of antagonistic potencies.

The solid-phase method of peptide synthesis (*7*), as adapted for the synthesis of oxytocin (*8*), with previously described modifications (*2, 9*), was used to prepare the protected intermediates required for the synthesis of each analog. The paranitrophenyl active ester method (*10*) facilitated by hydroxybenzotriazole (*11*) was used for the coupling of β-(*S*-benzyl mercapto)-β,β-cyclopentamethylenepropionic acid (*12*) to give the respective immediate precursors of analogs **1** and **4**. Each precursor was deblocked with sodium in liquid ammonia (*13*) as previously described (*8, 9*), and the resulting disulfhydryl compounds were oxidatively cyclized with potassium ferricyanide (*14*). The analogs were desalted and purified by gel filtration on Sephadex G-15 by a two-step procedure (*15*) with 50 percent acetic acid and 0.2*M* acetic acid, respectively, as eluants. The purity and identity of each analog was ascertained by thin-layer chromatography in three different solvent systems (*2*) and by amino acid analysis (*16*).

When injected intravenously into ethanol-anesthetized, water-loaded female rats (*17*) these four analogs are weak antidiuretic agonists. They cause an initial submaximum inhibition of urine flow lasting about 10 minutes. This is followed by a period of inhibition of responses to ADH lasting 1 to 3 hours, depending upon dose. This inhibition is reversible in that it can be overcome by increasing the dose of ADH. The antagonistic potency of each analog was estimated from responses to the *United States Pharmacopeia* posterior pituitary reference standard injected 20 minutes after injection of antagonist. Two doses of an antagonist were injected into each rat: one dose that reduced the response to 2x units of standard to less than the response to 1x units before injection of antagonist and one that reduced the response but not to the level of the control response to 1x units. The "effective dose" was then obtained by interpolation on a logarithmic scale between the two doses of antagonist. It is defined as the dose of antagonist that would reduce the response to 2x units to match the control response to 1x units. This is the original method introduced by Schild (*18*) for estimating the effective concentrations of antagonists from which he derived pA_2 values as an index of antagonistic potency. The effective doses for these analogs estimated in this manner are presented in Table 1. The order of antagonistic potencies indicates that *O*-ethyltyrosine substitution is more effective than *O*-methyltyrosine in the 2-position, the two *O*-ethyltyrosine analogs (**2** and **4**) being more potent than their *O*-methyltyrosine counterparts (**1** and **3**). The presence of 8-D-arginine appears to be a disadvantage since the two L-arginine analogs (**3** and **4**) are more potent than the D-arginine analogs (**1** and **2**). From studies on a series of closely related analogs it is clear that the cyclopentamethylene group on the β-carbon of the 1-(β-mercaptopropionic acid) is also important. The corresponding β,β-diethyl and β,β-dimethyl analogs of analog **4** do not exert detectable antagonistic activity in the intravenous antidiuretic assay in the rat. The presence of the 4-valine also contributes to antagonistic activity. If a 4-glutamine is substituted in this position in analog **4**, antagonistic activity is lost.

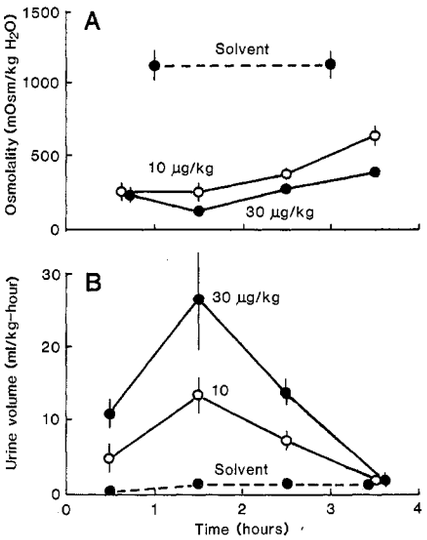

Fig. 2. Responses by intact rats to intraperitoneal injections of analog **4**, $d(CH_2)_5Tyr(Et)VAVP$. Rats were given free access to water and were injected at 11 a.m. Spontaneously voided urine was collected hourly for 4 hours. Six female rats, weighing 200 to 240 g, were used in a simple block design so that each rat received solvent and both doses of the antagonist. Injections were given at least 2 days apart. Urine osmolalities for the control (solvent-injected) rats are averaged over 2-hour periods because of the infrequency of urination. Vertical lines through points indicate standard errors.

These analogs can also antagonize endogenous ADH. When injected intraperitoneally into normally hydrated conscious female rats they cause a rapid increase in urine flow and a fall in urinary osmolality (Fig. 2). The order of relative antagonistic potencies of the analogs in this assay (Table 2) appears similar to the order of potencies estimated from intravenous injection into anesthetized rats (Table 1). The higher intraperitoneal doses appear to block almost completely the antidiuretic action of endogenous ADH. For example, the 30 μg/kg dose of analog **4** raised urine flow to a mean of 27 ml per kilogram per hour during the second hour after injection (Fig. 2). In female rats homozygous for the Brattleboro strain, which secrete no ADH at all, spontaneous urine flow averages 32 ml per kilogram per hour (*19*).

Table 2. Urine excretion and osmolality in 4 hours following the intraperitoneal injection of ADH antagonists into conscious rats. The figures shown are means ± standard error for groups of four or six rats.

Analog	Dose (μg/kg)	Urine volume (ml/kg-hour)	Osmolality (mOsm/kg H_2O)
1 $d(CH_2)_5Tyr(Me)V_DAVP$	100	1.5 ± 0.3	1341 ± 428
	300	2.2 ± 0.4*	961 ± 204†
2 $d(CH_2)_5Tyr(Et)V_DAVP$	30	2.8 ± 0.3†	640 ± 47†
	100	9.5 ± 1.8†	234 ± 25†
3 $d(CH_2)_5Tyr(Me)VAVP$	10	1.1 ± 0.5	1303 ± 190
	30	3.4 ± 0.9*	514 ± 105†
4 $d(CH_2)_5Tyr(Et)VAVP$	10	7.4 ± 1.0†	316 ± 38†
	30	13.3 ± 2.5†	194 ± 17†

*$P < .05$ and †$P < .005$ for differences between the means for rats receiving antagonists compared to the mean responses of the same rats injected with solvent only. The mean control rate for solvent-injected rats was 0.9 ± 0.1 ml/kg-hour and the mean osmolality was 1544 ± 85 mOsm per kilogram of water ($N = 32$).

These four analogs appear to be the most effective antagonists of the antidiuretic response to ADH yet reported. If these analogs have a similar action in man they could be useful pharmacologically for studies on the contribution that ADH may make in a variety of pathological states involving water retention. They could also be effective and specific agents for treating the syndrome of inappropriate secretion of ADH (the Schwartz-Bartter syndrome or SIADH). This syndrome may complicate a number of disorders, including carcinomas, pulmonary diseases, intracranial diseases, and head injuries (*20*).

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Chemical Impurity Produces Extra Compound Eyes and Heads in Crickets

Abstract. *A chemical impurity isolated from commercially purchased acridine causes cricket embryos to develop extra compound eyes, branched antennae, extra antennae, and extra heads. Purified acridine does not produce similar duplications of cricket heads or head structures nor do the substituted acridines proflavine, acriflavine, or acridine orange. A dose-response relation exists such that the number and severity of abnormalities increase with increasing concentration of the teratogen.*

While determining toxicity of acridine, an azaarene of interest as a component of wastes from coal gasification and liquefaction, a contaminant of the commercial product (*1*) was found to be teratogenic to the cricket *Acheta domesticus* (L.) (*2*) in minute amounts. Treatment of eggs resulted in duplication of cricket head structures. This finding underscores the necessity for complete chemical characterization of mixtures subjected to toxicological assays because a minor component can assume major significance in producing a response. In a more positive light, however, this teratogen offers possibilities as a new tool for exploring cellular events in embryonic development such as determination of cell fate, pattern formation, and possibly the role of cell death in morphogenesis.

Cricket eggs were exposed to commercial samples of acridine by permitting gravid females to oviposit in sand moistened with aqueous solutions of the compound (*3*). After the embryos were exposed to acridine, a small percentage developed extra compound eyes; this percentage increased with increasing concentrations of the commercial product (Table 1). Purification of the commercial product, which was initially > 98 percent acridine, resulted in complete loss of teratogenic activity, whereas the mixture of impurities showed a positive response (*4*). These impurities were obtained in quantities too small to weigh accurately; however, ten chemicals were isolated by preparative thin-layer chromatography on silica gel G plates (E. Merck) in a mixture of chloroform, methanol, and acetic acid (90:5:5, by volume). Each compound was assayed for teratogenicity to cricket eggs; and only one (R_F = 0.74), which showed blue fluorescence under long-wave ultraviolet light, was active (Table 1).

Speculation about the chemical identity of the active impurity is difficult since the method of synthesis is not available from the manufacturer. Many substituted acridines, however, bind nucleic acids (*5*) and are active bactericides, antimalarial and antineoplastic agents, mutagens, and carcinogens (*6*). Three of these, proflavine (3,6-diaminoacridine hydrochloride), acridine orange [3,6-bis-(dimethylamino)acridine hydrochloride], and acriflavine hydrochloride (a mixture of the hydrochlorides of 3,6-diamino-10-methylacridinium chloride and 3,6-diaminoacridine), were evaluated for toxicity to eggs and ability to produce multiple-eyed crickets. None was as toxic to the eggs as pure acridine (Table 1, LC_{50} data), and none produced dupli-

Table 1. Toxicity and teratogenic activity of various chemicals to developing cricket embryos.

Treatment	LC_{50} (ppm)	Concentration (ppm)	Eggs (No.)	Embryos with abnormal number of eyes	
				No.	%
Distilled water			30,153	0	
Impure acridine		20	2,264	28	1.24
		9	2,234	10	0.44
		6	4,802	7	0.14
Purified acridine	15.1 ± 0.61*	20	3,214	0	
		9	4,337	0	
		6	5,123	0	
Acridine impurities			3,728	212	5.69
Unknown (R_F, 0.74)			2,681	25	0.93
Acriflavine	> 100	100	7,817	0	
		10	6,645	0	
Proflavine	> 100	100	5,056	0	
		10	4,343	0	
Acridine orange	> 100	100	2,627	0	
		10	2,372	0	

*From Walton (*14*).

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