

## Suppression of Natriferic Activity of the Vasopressin Molecule by Modifications in Positions 1, 2 and 4

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**Abstract.** The capacity of five synthetic analogs of [8-arginine] vasopressin (AVP) to stimulate frog skin sodium transport (natriferic activity) was characterized electrophysiologically using the method of short-circuit current, and compared to that of synthetic AVP. The analogs used were [8-arginine] vasopressins modified in positions 1 and 2: [1-(1-mercapto-4-tert-butylcyclohexaneacetic acid)] AVP (I); [1-(1-mercapto-4-methylcyclohexaneacetic acid)] AVP (II); [1-(1-mercapto-4-methylcyclohexaneacetic acid)-2-O-methyltyrosine] AVP (III); and in position 4: [1-(1-mercaptocyclohexaneacetic acid)-4-arginine] AVP (IV); [1-(2-mercaptopropionic acid)-4-arginine] AVP (V). The addition of synthetic vasopressins I, II and V to the frog skin resulted in a weaker stimulation of the skin sodium transport, measured as the level of the short-circuit current ( $I_{sc}$ ), as compared to that induced by synthetic AVP. In relation to natriferic activity, analogs III and IV did not change the electrical parameters of the skin. It is concluded that introduction of cyclic structure at the  $\beta$ -carbon in position 1 of the vasopressin molecule decreased its natriferic activity by about 70%. The same reduction of the activity was caused by the replacement of the glutamine residue in position 4 with arginine, and deamination in position 1. Cyclic structure bound in position 1 together with methylation of tyrosine in position 2 resulted in a full suppression of natriferic activity. Similarly, introduction of cyclic group in position 1 in combination with substitution of glutamine in position 4 with arginine totally abolished natriferic activity.

**Key words:** Arginine-vasopressin — Synthetic analogs — Natriferic activity — Frog skin

### Introduction

Arginine-vasopressin (AVP) interacts in the periphery primarily with two distinct receptor types designated  $V_1$  and  $V_2$  (Michell et al. 1979).  $V_1$  receptors in

vascular smooth muscle (Penit et al. 1983) and on hepatocytes (Keppens and De Wulf 1979) modulate vasopressor and glycogenolytic responses to AVP by a  $\text{Ca}^{2+}$ -dependent pathway (Michell et al. 1979).  $V_2$  receptors on renal tubules modulate the antidiuretic responses to AVP by a cyclic adenosine monophosphate (cAMP)-dependent pathway (Butlen et al. 1978).

It has been established that structural modifications of the vasopressin molecule can affect its receptor affinity and efficacy. Since the original synthesis of arginine-vasopressin in 1954 by du Vigneaud et al., hundreds of selective  $V_1$  and  $V_2$  agonists and antagonists of AVP have been synthesized (Manning et al. 1987; 1988). Examination of the ligand selectivity of the  $V_1$  receptors in an increasing number of tissues revealed striking similarities between these receptors. Thus, it would appear that a large number of AVP actions are mediated by the same subtype of receptor. In the past few years a new type of vasopressin receptor was described in the anterior pituitary. This novel subtype has been designated  $V_3$  or  $V_{1h}$ , as distinct from the vascular and hepatic receptors which have been termed  $V_{1a}$  receptors (Jard et al. 1986). This receptor type has some attributes of both the  $V_1$  (pressor) and  $V_2$  (antidiuretic) receptor.

The present work was aimed at testing the natriuretic activity of synthetic analogs of AVP, modified in positions 1 and 2: [1-(1-mercapto-4-tert-butylcyclohexaneacetic acid)] AVP (I), [1-(1-mercapto-4-methylcyclohexaneacetic acid)] AVP (II), [1-(1-mercapto-4-methylcyclohexaneacetic acid)-2-O-methyltyrosine] AVP (III), synthesized by Lammek et al. (1988); and modified in position 4: [1-(1-mercapto-4-tert-butylcyclohexaneacetic acid)-4-arginine] AVP (IV), [1-(2-mercapto-4-tert-butylcyclohexaneacetic acid)-4-arginine] AVP (V), synthesized by Rekowski et al. (1985). The activities of these analogs were compared to that of AVP. It was shown that modifications in positions 1, 2 and 4 of the vasopressin molecule result in inhibition or complete suppression of its natriuretic activity.

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$V_{oc}$  — transepithelial potential difference under open-circuit conditions ( $I_{sc} = 0$ )

$I_{sc}$  — short-circuit current at zero transepithelial potential ( $V_{oc} = 0$ )

$R_t$  — transepithelial d.c. resistance ( $R_t = V_{oc}/I_{sc}$ )

## Materials and Methods

*Animals and experimental setup:* Experiments were performed throughout the year on isolated skins of European frogs (*Rana temporaria*). The frogs were kept at  $+5^\circ\text{C}$  until the day of experiment when they were pithed, the abdominal skin stripped and mounted vertically between two halves of an Ussing type chamber (area =  $1\text{ cm}^2$ ) with recirculating Ringer solution (Bentley 1958). To prevent electrical leaks and to cover up the possible damaged edges, the space between the skin and the

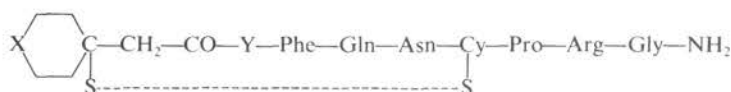
hemichambers was sealed by a thin layer of high-grade silicone grease. The volume of the chamber was 8 ml each. The experiments were performed at room temperature (20–22°C).

*Electronics:* In the experiments four-electrode automatic voltage-clamp with high input impedance input stage was used as described in details elsewhere (Ponec et al. 1989a; b). The skin preparations were short-circuited throughout the experiment with short interruptions to obtain a measure of the open-circuit transepithelial potential difference.

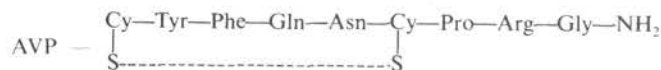
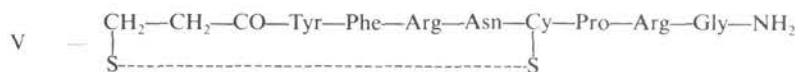
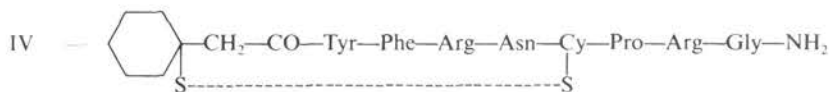
*Experimental protocol:* After mounting the isolated skin in the perfused chamber time was allowed until basal parameters stabilized. [8-arginine] vasopressin or its synthetic analogs (see Symbols and chemical composition of the analogs) were dissolved in Ringer solution and added in ascending concentrations (from  $10^{-7}$  to  $10^{-5}$  mol/l) to the solution bathing the basolateral frog skin surface, without changing osmolality, pH or volume of the bathing solution. Natriferic activity (capacity to stimulate sodium transport) of synthetic analogs was measured as the level of short-circuit current flowing through the skin.

*Calculations and statistics:* For calculation of transepithelial d.c. resistance Ohm's law formula was employed (Ussing and Zerahn 1951). Student's *t*-test was used for statistical analyses. Preparations tested: Synthetic analogs were synthesized at the Institute of Chemistry, University of Gdansk, Poland. The synthesis, basal pharmacological data, and some in vivo biological properties of the analogs were described elsewhere (Lammek et al. 1988; Rekowski et al. 1985). Synthetic [8-arginine] vasopressin was kindly provided by Dr. T. Barth, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague. Symbols and chemical composition of the analogs:

- I — [1-(1-mercapto-4-tert-butylcyclohexaneacetic acid)] AVP  
 II — [1-(1-mercapto-4-methylcyclohexaneacetic acid) AVP  
 III — [1-(1-mercapto-4-methylcyclohexaneacetic acid)-2-O-methyltyrosine] AVP  
 IV — [1-(1-mercapto-4-methylcyclohexaneacetic acid)-4-arginine] AVP  
 V — [1-(2-mercapto-4-methylcyclohexaneacetic acid)-4-arginine] AVP  
 AVP — [8-arginine] vasopressin



- I — X = CHC(CH<sub>3</sub>)<sub>3</sub>; Y = Tyr  
 II — X = CHCH<sub>3</sub>; Y = Tyr  
 III — X = CHCH<sub>3</sub>; Y = Tyr (Me)



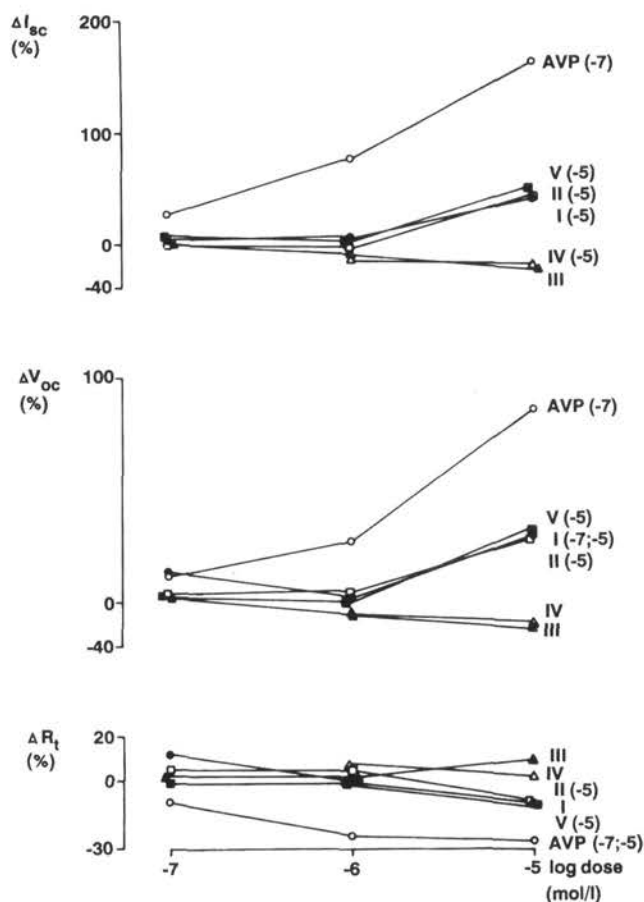


Fig. 1. Dose dependences of the effects of AVP and its synthetic analogs (for symbols see Materials and Methods), expressed as percentage differences of the original values, upon the short-circuit current ( $I_{sc}$ ), transepithelial electrical potential ( $V_{oc}$ ) and transepithelial electrical resistance ( $R_t$ ) of the frog skin. Values in brackets show molar concentrations of the hormone preparations tested, at which the effects were statistically significant. Significance level:  $p < 0.05$ .

## Results

### Transport parameters

$[I_{sc}]$  — [8-arginine] vasopressin and its synthetic analogs I, II and V (for symbols see Materials and Methods) applied to the basolateral side of the frog skin



**Table 1.** Comparison of the effect of AVP with those of its synthetic analogs I, II and V, on the frog skin sodium transport parameters expressed as percentage differences between the basal and stimulated levels ( $\Delta\%$ ). For symbols see Introduction; Materials and Methods. Asterisks denote statistically significant changes in relation to AVP. Significance level:  $p < 0.05$ . Means  $\pm$  S.E.M.

Con- centra- tion [mol/l]	AVP		Analog I		Analog II		Analog V	
		<i>n</i>		<i>n</i>		<i>n</i>		<i>n</i>
$\Delta I_{sc}$ [%]								
$10^{-7}$	24.4 $\pm$ 3.5	5	3.2 $\pm$ 7.5*	5	0 $\pm$ 0.0*	6	4.9 $\pm$ 2.9*	8
$10^{-6}$	78.8 $\pm$ 24.5	6	5.8 $\pm$ 7.1*	6	-1.1 $\pm$ 3.1*	9	2.0 $\pm$ 2.4*	8
$10^{-5}$	165.0 $\pm$ 37.9	8	41.4 $\pm$ 5.7*	8	42.0 $\pm$ 11.5*	8	48.3 $\pm$ 9.9*	7
$\Delta V_{oc}$ [%]								
$10^{-7}$	12.8 $\pm$ 2.6	5	14.6 $\pm$ 5.7	5	4.0 $\pm$ 1.1*	6	2.8 $\pm$ 4.3	8
$10^{-6}$	27.0 $\pm$ 4.6	6	3.0 $\pm$ 3.0*	6	3.6 $\pm$ 2.3*	9	-0.1 $\pm$ 2.3*	8
$10^{-5}$	86.6 $\pm$ 23.2	8	29.0 $\pm$ 7.8*	8	29.0 $\pm$ 8.9*	8	31.9 $\pm$ 4.4*	7
$\Delta R_t$ [%]								
$10^{-7}$	-9.2 $\pm$ 1.9	5	13.2 $\pm$ 8.2*	5	4.0 $\pm$ 1.1*	6	-2.1 $\pm$ 4.1	8
$10^{-6}$	-24.7 $\pm$ 6.9	6	-0.3 $\pm$ 8.4*	6	5.1 $\pm$ 2.1*	9	-1.5 $\pm$ 2.0*	8
$10^{-5}$	-26.6 $\pm$ 5.4	8	-9.9 $\pm$ 4.5*	8	-9.0 $\pm$ 2.4*	8	-10.1 $\pm$ 3.1*	7

significantly stimulated the active sodium transport across the epithelium as reflected by increased levels of short-circuit current ( $I_{sc}$ ) (Fig. 1). After AVP, stimulation was observed already with the drug concentration of  $10^{-7}$  mol/l, whereas after the synthetic analogs (I, II, V) it occurred only at a concentration of  $10^{-5}$  mol/l. Thus, as compared with AVP, the synthetic analogs stimulated  $I_{sc}$  to a smaller degree (Fig. 1, Table 1). Analog III did not change the level of  $I_{sc}$ , and analog IV in a concentration of  $10^{-5}$  mol/l caused a slight current decrease.

[ $V_{oc}$ ] — AVP and its synthetic analogs I, II and V increased the level of the transepithelial potential difference ( $V_{oc}$ ), to similar degree (Fig. 1). The stimulation by AVP and its analogs was observed to occur at the same concentrations as observed for  $I_{sc}$ . The analogs-induced increase of  $V_{oc}$  was also smaller than that induced by AVP, similarly as was the case with  $I_{sc}$  (Fig. 1, Table 1). Analog III and IV did not change the level of  $V_{oc}$ .

[ $R_t$ ] — AVP, at concentrations of  $10^{-7}$  to  $10^{-5}$  mol/l decreased the transepithelial d.c. resistance ( $R_t$ ) of the frog skin (Fig. 1). After application of synthetic analogs II and V, at concentration of  $10^{-5}$  mol/l, only a small decrease of  $R_t$  was observed (Fig. 1, Table 1). Analog III and IV were ineffective in influencing  $R_t$ .

**Table 2.** Comparison of biological activities of synthetic vasopressins expressed as percentage differences in relation to synthetic [8-arginine] vasopressin (AVP)\*. For symbols see Materials and Methods.

Analog	Biological activities [% of AVP activity]			
	antidiuretic	pressor	natriferic	
			$10^{-6}$ mol/l	$10^{-5}$ mol/l
I	0.04	0.01	7.4	25.1
II	0.04	0.0	-1.4	25.5
III	0.21	0.0	-9.6	-9.9
IV	0.004	0.07	-14.4	-7.6
V	0.66	0.33	2.5	29.3

\* Values of natriferic activity, after application of analogs in concentrations of  $10^{-6}$  and  $10^{-5}$  mol/l, were recalculated and compared to data of antidiuretic and pressoric activities of AVP and its analogs reported by Manning et al. 1987 and Lammek et al. 1988. The activity of AVP was taken as reference value. A negative value of a change means that the analog acted in opposite direction.

## Discussion

The results of this in vitro study provide evidence for a decreased or no natriferic action of synthetic vasopressins, modified in positions 1, 2 and 4 of the molecule, in comparison with parent [8-arginine] vasopressin. The stimulation of the frog skin active sodium transport, as shown by both the increased short-circuit ( $I_{sc}$ ) and the increased transepithelial potential difference ( $V_{oc}$ ), was weaker with analogs I, II and V than with the parent molecule. Analog III and IV induced no statistically significant changes of these parameters.

It was shown earlier that modifications of the vasopressin molecule in positions 1 and 2, which are proposed to be the key positions of AVP antagonists, can convert a highly active antidiuretic and vasopressor agonist into a potent vasopressor antagonist (Kruszynski et al. 1980). Pharmacological data of vasopressin analogs obtained by this modification (analog I, II and III) show that none of them exhibit any measurable pressor agonistic activity, and all are potent antagonists of the vasopressor response to AVP. All analogs have low antidiuretic agonistic activities, which give them a high antivasopressor/antidiuretic selectivity (Lammek et al. 1988). It could be proposed based on our results that the decreased (analog I, II) and weak or inconsistent (III) natriferic activity measured in our in vitro experiments is in good agreement with the low antidiuretic activity observed by Lammek et al. (1988) in in vivo experiments (Table 2). The natriferic effect is then tightly matched with the antidiuretic effect

of the vasopressin molecule. The site where these two effects are materialized is the  $V_2$  receptor, as has already been described (Exton 1987; Manning et al. 1988). It is also clear that structural modifications of the vasopressin molecule can affect both its receptor affinity and efficacy. Our results showed that introduction of a cyclic structure at the  $\beta$ -carbon in position 1 (analogs I, II) was associated with decreased natriferic activity (to about 30%) in comparison with AVP (Table 2). Further methylation of this substance in position 2 (analog III) resulted in complete abolition of the natriferic activity. On the other hand, increased pressor antagonism associated with methylation of tyrosine was demonstrated also for the analogs with a large and more hydrophobic cyclic group in position 1 (Lammek et al. 1988).

The two other synthetic analogs were obtained by modifying the parent molecule in position 4: an additional arginine was substituted for the glutamine residue. The new analogs obtained were [1-(1-mercaptocyclohexaneacetic acid)-4-arginine] AVP (IV) and [1-(2-mercaptopropionic acid)-4-arginine] AVP (V). Earlier these synthetic vasopressins have been tested for their antidiuretic and pressor effects and the results were compared with that of synthetic [8-arginine] vasopressin. It was shown that analog V had decreased antidiuretic activity, whereas its pressor activity remained approximately constant (Rekowski et al. 1985). Analog IV had practically negligible effect in the antidiuretic assay, whereas its pressor activity was considerably higher than expected making this compound a pronounced pressor agonist rather than a potential pressor antagonist (Rekowski et al. (1985).

Tests of natriferic activity of the synthetic analogs studied showed a good accordance with antidiuretic activity. Analog V showed a decreased natriferic activity as compared to that of [8-arginine] vasopressin, whereas analog IV was ineffective in this respect (Table 2). This ineffectiveness could be also ascribed to the introduction of the cyclic group at the  $\beta$ -carbon in position 1. Replacement of the glutamine residue in the vasopressin molecule by additional arginine did not change its antidiuretic activity and decreased its pressor activity to about 40% (Rekowski et al. 1985). Further deamination of this substance in position 1 caused a drop in the antidiuretic activity to about 50% (Rekowski et al. 1985). It is important to note that deamination of the new complex resulted in a decreased instead of increased antidiuretic, in the case mentioned natriferic activity. There are discrepancies in biological and pharmacological properties of the new analogs obtained by deamination of the parent AVP molecule. It was shown earlier for dDAVP, (1-deamino-8-D-arginine-vasopressin), that deamination increases and prolongs the antidiuretic (Vávra et al. 1968) and natriferic (Bakoš et al. 1984) but weakens the hydroosmotic activity (Barth et al. 1975). Obviously, a single modification in the molecule can cause different changes in the biological activity of a new synthetic analog.



Studies, in which additional unknown characteristics of new synthetic analogs of hormones are tested, can provide further insight into the structure-function relationship. Synthetic analogs of [8-arginine] vasopressin, tested in the present work, have beside high antivasopressor and low antidiuretic activities, and their natriferic activities are low or fully suppressed. The inhibition of the natriferic effect by modification of the vasopressin molecule may be ascribed to partial agonism which was usually observed with similar preparations. It is known that the potency of an antagonist may vary considerably depending on the system and experimental conditions in which it is tested. Also, many antagonists have been shown not to be ideal agents since they preserve some intrinsic agonistic activities (Liard 1988).

Based on our results it is concluded that modifications in positions 1 and 2 of the vasopressin molecule by the introduction of a cyclic group at the  $\beta$ -carbon in position 1 and further methylation of tyrosine in position 2 caused a drop or complete abolition of the natriferic activity. Similarly, binding of the cyclic structure to position 1 and replacement of glutamine in position 4 by additional arginine also resulted in complete suppression of the natriferic activity. Exchange of additional arginine for glutamine and deamination of this complex caused decreased natriferic activity of the vasopressin molecule as compared to that of [8-arginine] vasopressin.

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