Hydroosmotic Activities of Arginine-Vasopressins Modified Either in Positions 1, 2 and 4 or at N-terminal Extensions

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Abstract. Vasopressin and its synthetic analogs were studied for their effect on transepithelial water flux in frog urinary bladder As compared with AVP, 1-deamino-8-D-arginine vasopressin (dDAVP) was about 40 times less effective in stimulating osmotic water flow The vasopressin analogs obtained by modification in positions 1 and 2 were [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 those modified in position 4 were [1-(1-mercaptocyclohexaneacetic acid)-4-arginine] AVP (IV), [1-(2-mercaptopropionic acid)-4-arginine]AVP (V) Any of the above analogs did not influence basal, but antagonized vasopressin-stimulated water flux N-terminally extended analogs of AVP Ala-AVP (VI), Ser-Ala-AVP (VII) and Thr-Ser-Ala-AVP (VIII) stimulated osmotic water flux to the same extent in concentration 200 times higher as that of AVP We conclude from these studies that vasopressin analogs (I-V) competitively antagonize vasopressin-stimulated hydroosmotic activity in frog urinary bladder probably at the epithelial vasotocin V_1 and/or V_2 receptor site N-terminal extension of the vasopressin molecule did not influence the capacity of AVP to induce V_2 receptor-mediated action, even when used at higher concentrations

Key words: Frog urinary bladder — Hydroosmotic activities — Arginine-vasopressin — Synthetic vasopressin agonists and antagonists

Introduction

Neurohypophyseal peptides have at least twofold effect on epithelial cells of amphibian skin and urinary bladder. They stimulate active sodium transport across the skin and increase osmotic water permeability of urinary bladder. The hydroosmotic action of vasopressin (AVP) has been thought to be mediated mainly through V_2 receptor activation that is coupled to adenylate cyclase (Handler and Orloff 1981). However, recent studies have shown that in renal epithelia, including amphibian urinary bladder, also V_1 vasopressin receptors are present (Yorio et al. 1981; Schlondorf and Satriano 1985). This receptor may play a role not only as a part of the negative feedback process, but also as an integral component of the enhanced water permeability mediated by vasopressin at the apical membrane (Yorio and Satumtira 1989). Using neurohypophyseal peptides, previous studies of structural requirements for activity on amphibian bladder epithelial cells (for review see Morel and Jard 1968) indicated that hydroosmotic activity is strongly affected by structural modifications of the hormone molecule. During the recent 10–15 years hundreds of selective V_1- and V_2-receptor agonists and AVP antagonists have been synthesized (for review see Manning et al. 1987; 1988; Manning and Sawyer 1989; Thibonnier 1990). Vasopressin analogs have been used as pharmacological and therapeutic tools in animal and human physiology and pathophysiology, and they play an important role in molecular pharmacology, contributing insight into the mechanism of antidiuretic activity and the nature of the antidiuretic receptors (Butlen et al. 1978; Stassen et al. 1984, 1985).

The present work was aimed at testing the hydroosmotic activities of synthetic analogs of AVP, with modifications in positions 1, 2 (Lammek et al. 1988) and 4 (Rekowski et al. 1985) of the vasopressin molecule, and N-terminally extended arginine-vasopressins (Lammek et al. 1987). Previous physiological and pharmacological studies of analogs obtained by modifications in positions 1 and 2 showed that all are potent antagonists of the vasopressor response to AVP (Lammek et al. 1988), while analogs with changed position 4 had changed antidiuretic/pressor ratio as compared with that of synthetic AVP (Rekowski et al. 1985). In our previous experiments on frog skin also suppressed natriferic activities (ability to stimulate active sodium transport) of these analogs have been observed (Bakoš et al. 1990a). Synthesis of three N-terminally extended vasopressins with high antidiuretic activity *in vivo* and prolonged effect as compared to original AVP was reported by Lammek et al. (1987). This extension had no influence on the natriferic properties of AVP as for both the magnitude and the duration of action (Ponec et al. 1990).

The results of the present study indicate that modifications in positions 1, 2 and 4 of the AVP molecule strongly inhibit its basal hydroosmotic activity, and expressively suppress or block AVP-stimulated osmotic water flow. On the other hand, N-terminal extension of the vasopressin molecule causes total diminishion of

its hydroosmotic activity, whereas it does not influence the capacity to stimulate hydroosmotic activity at high concentrations.

Materials and Methods

Animals and experimental setup: Experiments were performed within January – June on isolated urinary bladders of European frogs, *Rana temporaria*, of either sex. The animals were kept at +5 °C until the day of experiments when they were pithed and the hemibladders excised. These were then prepared as sacs. Osmotic water movement was measured gravimetrically in the presence of an osmotic gradient using the modified method of Natochin and Shakhmatova (1966). This method consists of bathing the serosal side of the bladder with aerated Ringer's solution (in $mmol.l^{-1}$: 115 NaCl; 2.5 $KHCO_3$; 1 $CaCl_2$; pH 7.8; osmolality 225 $mosm.kg^{-1}$ H_2O). The osmotic gradient was established by diluting 1:10 the mucosal Ringer's solution. Water flow was measured in paired hemibladder sacs in the presence and absence of agents tested. Always one of the paired sacs served as a control. This protocol was adopted to eliminate inter-individual differences.

Experimental protocol: After preparing and placing the isolated urinary bladders into Ringer's solution, 30–40 min were allowed until basal water flow stabilized. [Arg8]-vasopressin or its synthetic analogs (see Reagents) were dissolved in Ringer's solution and added always in ascending concentrations (from 10^{-9} to 10^{-6} $mol.l^{-1}$) to the solution bathing the serosal surface of the bladder without changing osmolality, pH or volume of the bathing solution. Hydroosmotic activity (water flow, $\mu l.cm^{-2}.min^{-1}$) was measured as the decrease in sac weight at the end of each 30 min incubation period. Sacs were blotted gently on gauze before each weighing procedure.

Preparations tested: [Arg8]-vasopressin and its 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. 1987, 1988, Rekowski et al. 1985). [1-Deamino-8-D-arginine] vasopressin (dDAVP, V_2-agonist of AVP) was kindly provided by Dr. Barth, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague. Selective V_2-antagonist of AVP was obtained from Prof M. Manning, Department of Biochemistry, Medical College of Ohio, USA.

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-mercaptocyclohexaneacetic acid)-4-arginine]AVP
V – [1-(2-mercaptopropionic acid)-4-arginine]AVP
VI – Ala-AVP
VII – Ser-Ala-AVP
VIII – Thr-Ser-Ala-AVP
dDAVP – [1-Deamino-8-D-arginine] vasopressin
V_2-ANT – $d(CH_2)_5$[D-Ile^2, Ile^4, Ala^9-NH_2]AVP
AVP – [Arg8]-vasopressin

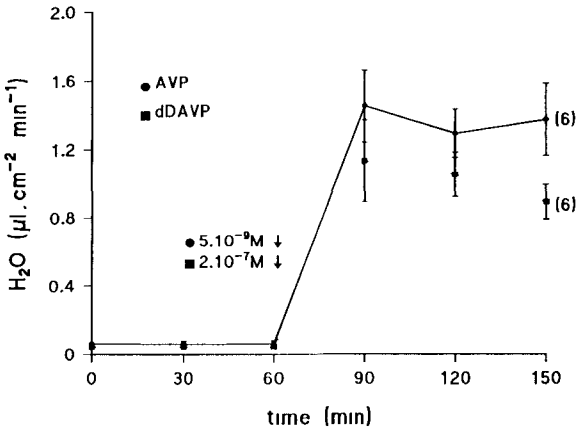

Figure 1. Comparison of the stimulatory effects of AVP and its synthetic analog, dDAVP, on water permeability in frog urinary bladder. Solid line: time-course of AVP effect; interrupted line: effect of dDAVP. For symbols see Materials and Methods. Values in the brackets indicate the number of bladders tested. Means ± S.E.M.

Results

Comparison of the effects of AVP and dDAVP

In the first series of experiments we compared the effects of [Arg8]-vasopressin (AVP) and its synthetic analog [1-deamino-8-D-arginine] vasopressin (dDAVP) on the hydroosmotic activity of frog urinary bladder (Table 1). AVP at 5.10^{-9}mol.l^{-1} expressively stimulated basal hydroosmotic activity of the bladder (Table 1*a*), (Fig. 1). The magnitude of this stimulation was independent of the sex of the experimental animals. At 40 times higher concentration (2.10^{-7}mol.l^{-1}) stimulatory effect of V_2-receptor agonist, dDAVP, similar to that of AVP was recorded (Table 1*b*; Fig. 1). At a lower concentration (4.10^{-8}mol.l^{-1}) dDAVP had no effect on hydroosmotic activity (Table 1*b*). Additional AVP application at the maximal dDAVP response did not cause statistically significant increase in hydroosmotic activity (Table 1*c*; Fig. 2). Agonistic activity of dDAVP was tested by using selective V_2-receptor antagonist (See Materials and Methods) with the stimulatory effect of dDAVP on osmotic water flow being completely suppressed (Table 1*c*; Fig. 2).

Modifications of the Arg8-vasopressin molecule in positions 1, 2 and 4

In another series of experiments we tested the hydroosmotic activities of synthetically prepared analogs of AVP (for symbols, chemical composition and other properties see Introduction and Materials and Methods). Effect of synthetic analog I is summarized in Table 2. In lower concentrations (10^{-9}–10^{-8} mol.l^{-1}) it had no influence upon basal water uptake. After additional application of AVP (5.10^{-9}

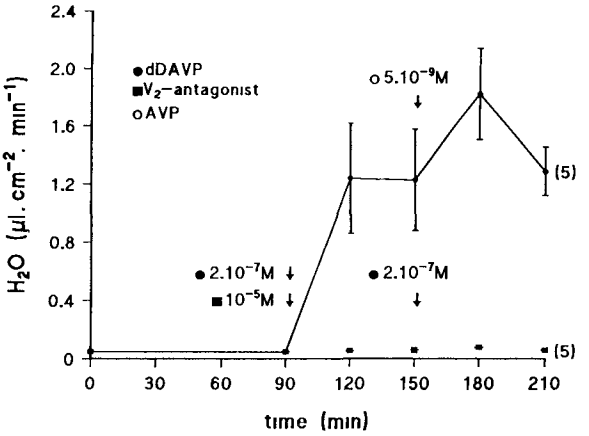

Figure 2. Test of aditivity of dDAVP and AVP application (AVP was applied at the maximal dDAVP response – solid line) and agonistic activity of dDAVP (after pretreatment with dDAVP, a selective V_2-receptor antagonist was added – interrupted line). For symbols see legend to Fig. 1.

mol.l^{-1}) the uptake strongly increased similarly as after AVP application alone (Table 2*a*). At a higher analog concentration (10^{-5}mol.l^{-1}) no effect on basal osmotic water flow was recorded either, but the stimulation level after additional AVP application was strongly inhibited (Table 2*b*, *c*; Fig 3). Inhibition of stimulation after pretreatment with analog I (10^{-5}mol.l^{-1}) was obtained also with dDAVP (Table 2*d*)

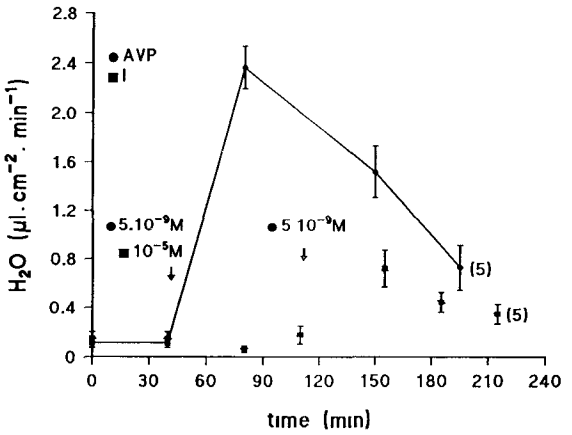

Figure 3. Comparison of the effects of AVP and analog I on the hydroosmotic activity of frog urinary bladder cells. Solid line: effect of AVP; interrupted line: effect of AVP after pretreatment of bladders with analog I. The Figure illustrates the effects of synthetic analogs I – V. For symbols see legend to Fig. 1.

Table 1. Comparison of the effects of AVP and dDAVP on frog urinary bladder wall permeability for water. Isoosmotic Ringer's solution (mmol.l^{-1}: Na 108, K 2.9; hypotonic Ringer's solution: Na 11, K 0.3). 1 – 5 : Wall permeability determinations in consecutive 30–40 min intervals. In experiments (a, b, c) AVP and dDAVP were applied always to the serosal surface of the bladder in various intervals as defined above (INT). Mean ± S.E.M. n = number of experiments. Significance as compared to the respective control of the experimental group (Interval 1): *; between two experimental groups: **.

agents		dose	n	int	water uptake (μl.cm^{-2}.min^{-1})				
		mmol.l^{-1}			1	2	3	4	5
a	AVP	$5 \cdot 10^{-9}$	6	2	0.06 ± 0.02	$1.45 \pm 0.23^{*}$	$1.30 \pm 0.14^{*}$	$1.34 \pm 0.21^{*}$	
	dDAVP	$2 \cdot 10^{-7}$	6	2	0.04 ± 0.01	$1.13 \pm 0.24^{*}$	$1.05 \pm 0.13^{*}$	$0.89 \pm 0.11^{*}$	
b	AVP	$5 \cdot 10^{-9}$	8	2	0.07 ± 0.02	$1.38 \pm 0.25^{*}$	$1.14 \pm 0.20^{*}$	$1.14 \pm 0.20^{*}$	
	dDAVP +	$4 \cdot 10^{-8}$	10	2	0.05 ± 0.02	0.12 ± 0.03			
	dDAVP	$2 \cdot 10^{-7}$		3			$0.54 \pm 0.11^{**}$	$1.09 \pm 0.21^{*}$	
c	dDAVP +	$2 \cdot 10^{-7}$	5	2	0.05 ± 0.01	1.24 ± 0.38	1.23 ± 0.35		
	AVP	$5 \cdot 10^{-9}$		4				1.83 ± 0.32	1.29 ± 0.26
	V2-ANT +	10^{-5}	5	2	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.02		
	dDAVP	$2 \cdot 10^{-7}$		4				0.08 ± 0.02	0.06 ± 0.01

Table 2. Effect of analog I on the frog urinary bladder wall permeability for water and its comparison to that of AVP and dDAVP. For symbols see legend to Table 1.

agents		dose	n	int	water uptake ($\mu l.cm^{-2}.min^{-1}$)				
		$mmol.l^{-1}$			1	2	3	4	5
a	AVP	$5 \cdot 10^{-9}$	8	2	0.08 ± 0.01	$1.75 \pm 0.27^{*}$	$1.65 \pm 0.24^{*}$	$1.15 \pm 0.18^{*}$	
	I	10^{-9}	8	2	0.07 ± 0.02	$0.10 \pm 0.01^{**}$			
	+AVP	$5 \cdot 10^{-9}$		3			$1.91 \pm 0.28^{*}$	$1.55 \pm 0.22^{*}$	
b	AVP	$5 \cdot 10^{-9}$	4	2	0.07 ± 0.01	$1.66 \pm 0.14^{*}$	$1.60 \pm 0.13^{*}$	$1.24 \pm 0.04^{*}$	$1.30 \pm 0.17^{*}$
	I	10^{-5}	5	2	0.06 ± 0.01	$0.08 \pm 0.02^{**}$	$0.07 \pm 0.02^{**}$	$0.09 \pm 0.02^{**}$	$0.12 \pm 0.03^{**}$
c	AVP	$5 \cdot 10^{-9}$	5	2	0.11 ± 0.04	$2.36 \pm 0.17^{*}$	$1.52 \pm 0.21^{*}$	$0.73 \pm 0.18^{*}$	
	I	10^{-5}	5	2	0.15 ± 0.05	$0.06 \pm 0.03^{**}$	$0.18 \pm 0.07^{**}$		
	+AVP	$5 \cdot 10^{-9}$	5	4				$0.72 \pm 0.15^{*}$	$0.45 \pm 0.08^{*}$
d	dDAVP	10^{-7}	8	2	0.07 ± 0.02	$1.33 \pm 0.28^{*}$	$0.74 \pm 0.16^{*}$	$0.82 \pm 0.20^{*}$	
	I	10^{-5}	7	2	0.08 ± 0.01	$0.07 \pm 0.02^{**}$	$0.05 \pm 0.01^{**}$		
	+dDAVP	10^{-7}		4				0.40 ± 0.25	0.24 ± 0.12

Table 3. Effect of analog II on the frog urinary bladder wall permeability for water and its comparison to that of AVP and dDAVP For symbols see legend to Table 1

agents		dose	n	int	water uptake (μl cm^{-2} min^{-1})				
		mmol l^{-1}			1	2	3	4	5
a	AVP	5 10^{-9}	7	2	0 09 ± 0 02	1 46 ± 0 36*	1 28 ± 0 57*	0 92 ± 0 24*	
	II	10^{-9}	8	2	0 05 ± 0 01	0 10 ± 0 02**			
	+AVP	5 10^{-9}		3			1 91 ± 0 27*	1 55 ± 0 19*	
b	AVP	5 10^{-9}	8	2	0 05 ± 0 01	1 12 ± 0 21*	1 21 ± 0 18*	1 05 ± 0 16*	0 81 ± 0 08*
	II	2 10^{-5}	9	2	0 05 ± 0 01	0 05 ± 0 01**	0 09 ± 0 02**		
	+AVP	5 10^{-9}		4				0 07 ± 0 02**	0 11 ± 0 02**
c	dDAVP	10^{-7}	8	2	0 09 ± 0 01	0 50 ± 0 13*	0 51 ± 0 13*	0 61 ± 0 18*	
	II	10^{-5}	8	2	0 09 ± 0 01	0 09 ± 0 01**	0 07 ± 0 02**		
	+dDAVP	10^{-7}		4				0 14 ± 0 04**	0 15 ± 0 05

Table 4. Effect of analog III on the frog urinary bladder wall permeability for water and its comparison to that of AVP and dDAVP. For symbols see legend to Table 1.

	agents	dose $mmol.l^{-1}$	n	int	water uptake ($\mu l.cm^{-2}.min^{-1}$)				
					1	2	3	4	5
a	AVP	$5 \cdot 10^{-9}$	6	2	0.08 ± 0.02	$1.07 \pm 0.27^{*}$	$1.09 \pm 0.26^{*}$	$0.85 \pm 0.19^{*}$	
	III	10^{-9}	6	2	0.06 ± 0.01	$0.07 \pm 0.01^{**}$			
	+AVP	$5 \cdot 10^{-9}$		3			$1.40 \pm 0.32^{*}$	$1.17 \pm 0.25^{*}$	
b	AVP	$5 \cdot 10^{-9}$	8	2	0.09 ± 0.02	$0.68 \pm 0.04^{*}$	$0.79 \pm 0.07^{*}$	$0.82 \pm 0.07^{*}$	$0.90 \pm 0.10^{*}$
	III	10^{-7}	7	2	0.11 ± 0.01	$0.09 \pm 0.01^{**}$			
	+AVP	$5 \cdot 10^{-9}$		3			$0.62 \pm 0.10^{*}$	$0.76 \pm 0.11^{*}$	$0.79 \pm 0.13^{*}$
c	AVP	$5 \cdot 10^{-9}$	7	2	0.07 ± 0.02	$1.41 \pm 0.21^{*}$	$1.18 \pm 0.17^{*}$	$1.03 \pm 0.14^{*}$	
	III	10^{-7}	7	2	0.08 ± 0.03	$0.09 \pm 0.03^{**}$	$0.07 \pm 0.02^{**}$		
	+AVP	$5 \cdot 10^{-9}$		4				$0.96 \pm 0.13^{*}$	$0.83 \pm 0.11^{*}$
d	AVP	$5 \cdot 10^{-9}$	7	2	0.06 ± 0.01	$1.09 \pm 0.19^{*}$	$1.32 \pm 0.20^{*}$	$0.99 \pm 0.17^{*}$	$0.84 \pm 0.11^{*}$
	III	$2 \cdot 10^{-6}$	6	2	0.07 ± 0.01	$0.07 \pm 0.01^{**}$	$0.09 \pm 0.02^{**}$		
	+AVP	$5 \cdot 10^{-9}$		4				$0.07 \pm 0.02^{**}$	$0.11 \pm 0.02^{**}$
e	AVP	$5 \cdot 10^{-9}$	6	2	0.13 ± 0.03	$1.42 \pm 0.21^{*}$	$1.52 \pm 0.22^{*}$	$1.14 \pm 0.14^{*}$	$0.89 \pm 0.13^{*}$
	III	10^{-5}	6	2	0.08 ± 0.02	$0.10 \pm 0.02^{**}$	$0.12 \pm 0.03^{**}$	$0.12 \pm 0.03^{**}$	
	+AVP	$5 \cdot 10^{-9}$		5					$0.36 \pm 0.17^{**}$
f	dDAVP	$2 \cdot 10^{-7}$	6	2	0.04 ± 0.01	$0.88 \pm 0.19^{*}$	$0.90 \pm 0.22^{*}$	$0.62 \pm 0.13^{*}$	$0.42 \pm 0.08^{*}$
	III	$2 \cdot 10^{-6}$	8	2	0.04 ± 0.01	$0.06 \pm 0.01^{**}$	$0.06 \pm 0.02^{**}$		
	+dDAVP	$2 \cdot 10^{-7}$		4				$0.11 \pm 0.03^{**}$	$0.14 \pm 0.04^{**}$

Table 5. Effects of analogs IV and V on the frog urinary bladder wall permeability for water and their comparison to that of AVP For symbols see legend to Table 1

agents		dose mmol l^{-1}	n	int	water uptake ($\mu l\ cm^{-2}\ min^{-1}$)				
					1	2	3	4	5
a	AVP	$5\ 10^{-9}$	6	2	$0\ 10 \pm 0\ 01$	$1\ 18 \pm 0\ 29^{*}$	$0\ 96 \pm 0\ 22^{*}$	$0\ 74 \pm 0\ 15^{*}$	$0\ 67 \pm 0\ 15^{*}$
	IV	10^{-5}	8	2	$0\ 13 \pm 0\ 03$	$0\ 11 \pm 0\ 02^{**}$	$0\ 13 \pm 0\ 03^{**}$	$0\ 11 \pm 0\ 03^{**}$	
	+AVP	$5\ 10^{-9}$		5					$0\ 15 \pm 0\ 04^{**}$
b	AVP	$5\ 10^{-9}$	8	2	$0\ 07 \pm 0\ 01$	$1\ 24 \pm 0\ 24^{*}$	$1\ 22 \pm 0\ 17^{*}$	$1\ 14 \pm 0\ 14^{*}$	$0\ 78 \pm 0\ 11^{*}$
	V	10^{-6}	9	2	$0\ 07 \pm 0\ 01$	$0\ 10 \pm 0\ 02^{*}$	$0\ 12 \pm 0\ 03^{**}$		
	+V	$3\ 10^{-6}$		4				$0\ 12 \pm 0\ 04^{**}$	$0\ 15 \pm 0\ 03^{**}$
c	AVP	$5\ 10^{-9}$	5	2	$0\ 08 \pm 0\ 01$	$1\ 04 \pm 0\ 32^{*}$	$1\ 07 \pm 0\ 25^{*}$	$0\ 99 \pm 0\ 21^{*}$	$0\ 88 \pm 0\ 11^{*}$
	V	10^{-5}	6	2	$0\ 09 \pm 0\ 02$	$0\ 10 \pm 0\ 03^{**}$	$0\ 11 \pm 0\ 02^{**}$	$0\ 12 \pm 0\ 04^{**}$	$0\ 12 \pm 0\ 04^{**}$
d	AVP	$5\ 10^{-9}$	3	2	$0\ 22 \pm 0\ 18$	$2\ 41 \pm 0\ 09^{*}$	$1\ 73 \pm 0\ 04^{*}$	$1\ 07 \pm 0\ 08^{*}$	
	V	10^{-5}	4	2	$0\ 13 \pm 0\ 06$	$0\ 19 \pm 0\ 08^{**}$	$0\ 19 \pm 0\ 07^{**}$		
	+AVP	$5\ 10^{-9}$		4				$1\ 07 \pm 0\ 32^{*}$	$1\ 15 \pm 0\ 30$

Table 6. Effects of analogs VI, VII and VIII on the frog urinary bladder wall permeability for water and their comparison to that of AVP. For symbols see legend to Table 1.

agents		dose	n	int	water uptake ($\mu l.cm^{-2}.min^{-1}$)				
		$mmol.l^{-1}$			1	2	3	4	5
a	AVP	$5 \cdot 10^{-9}$	7	2	0.06 ± 0.02	$1.24 \pm 0.26^{*}$	$1.09 \pm 0.20^{*}$		
	VI	$2 \cdot 10^{-8}$	9	2	0.06 ± 0.02	$0.10 \pm 0.03^{**}$			
	+VI	$2 \cdot 10^{-7}$		3			$0.09 \pm 0.03^{**}$		
b	AVP	$5 \cdot 10^{-9}$	6	2	0.06 ± 0.02	$1.47 \pm 0.35^{*}$	$1.16 \pm 0.19^{*}$	$1.15 \pm 0.16^{*}$	$0.68 \pm 0.12^{*}$
	VI	10^{-6}	6	2	0.05 ± 0.02	$0.59 \pm 0.14^{**}$	$1.02 \pm 0.19^{**}$	$1.33 \pm 0.22^{*}$	$1.24 \pm 0.14^{**}$
c	AVP	$5 \cdot 10^{-9}$	9	2	0.06 ± 0.01	$1.42 \pm 0.28^{*}$	$1.51 \pm 0.22^{*}$		
	VII	$2 \cdot 10^{-8}$	10	2	0.05 ± 0.01	$0.08 \pm 0.01^{**}$			
	+VII	$3 \cdot 10^{-7}$		3			$0.50 \pm 0.12^{**}$		
d	AVP	$5 \cdot 10^{-9}$	6	2	0.08 ± 0.02	$0.99 \pm 0.14^{*}$	$0.92 \pm 0.14^{*}$	$0.98 \pm 0.16^{*}$	$0.69 \pm 0.12^{*}$
	VII	10^{-6}	6	2	0.11 ± 0.03	$1.08 \pm 0.29^{*}$	$1.65 \pm 0.22^{**}$	$1.44 \pm 0.25^{*}$	$0.66 \pm 0.24^{*}$
e	AVP	$5 \cdot 10^{-9}$	8	2	0.06 ± 0.01	$1.34 \pm 0.16^{*}$	$1.27 \pm 0.19^{*}$	$1.14 \pm 0.11^{*}$	$0.63 \pm 0.07^{*}$
	VIII	10^{-6}	8	2	0.05 ± 0.01	$0.62 \pm 0.21^{**}$	$1.21 \pm 0.22^{*}$	$1.32 \pm 0.15^{*}$	$1.12 \pm 0.10^{**}$

The results of application of analog II are in Table 3. Lower concentrations changed neither basal nor AVP-stimulated hydroosmotic activity of the bladder (Table 3*a*). On the other hand, pretreatment with high doses of the analog effectivelly blocked the stimulatory effect of AVP (Table 3*b*) and/or dDAVP (Table 3*c*).

Table 4 shows the results obtained after addition of analog III. Neither this analog used in lower doses (10^{-9}-10^{-7}mol.l^{-1}) did change basal and AVP-stimulated osmotic water flow (Table 4*a*, *b*, *c*). This lack of effect was independent of the period of pretreatment with the analog tested. At 2.10^{-6}mol.l^{-1}, the analog strongly inhibited or effectively blocked the stimulatory effects of AVP and dDAVP (Table 4*d*, *e*, *f*).

Similar effects were observed after the application of high doses (10^{-5}mol.l^{-1}) of analogs IV (Table 5*a*) and V (Table 5*b*, *c*, *d*). The basal level of water uptake was not influenced, but the AVP-stimulated one was almost entirely suppressed (analog IV) or inhibited (analog V). However, as compared to the previous results, pretreatment with analog V caused less inhibition in the AVP- stimulated hydroosmotic activity (Table 5*d*).

N-terminally extended Arg8-vasopressins

In this series of experiments we tested the hydroosmotic activity of three N-terminally extended [Arg8]-vasopressins: Ala-AVP (VI), Ser-Ala-AVP (VII) and Thr-Ser-Ala-AVP (VIII). Results are summarized in Table 6.

Synthetic analog VI applied at 2.10^{-8} mol.l^{-1} and 2.10^{-7}mol.l^{-1} to the serosal side of the epithelium did not change the basal level of the frog urinary bladder hydroosmotic activity (Table 6*a*). Identical stimulation of this activity in comparison with AVP (5.10^{-9}mol.l^{-1}) appeared, however, only after application of a 200 times higher dose (10^{-6}mol.l^{-1}) (Table 6*b*; Fig. 4). Differences were detected also in dynamics of action. While AVP reached its maximal effect after 20–30 min, the synthetic analog needed about 60–80 min for maximal stimulation of hydroosmotic activity (Table 6*b*; Fig. 4).

Stimulation of osmotic water flow was also observed in the presence of high dose (10^{-6}mol.l^{-1}) of other two synthetic analogs, (VII) and (VIII). At this concentration both Ser-Ala-AVP and Thr-Ser-Ala-AVP increased the basal level of water uptake similarly as did AVP (Table 6*d*, *e*). The stimulatory effect of analog VII on osmotic water flow was even stronger as compared to that of AVP (Table 6*d*). Lower concentrations of the analogs tested were ineffective (Table 6*a*) or only a small increase in osmotic water flow could be observed (Table 6*c*). The time course of analog VIII action was changed and the maximal response was postponed.

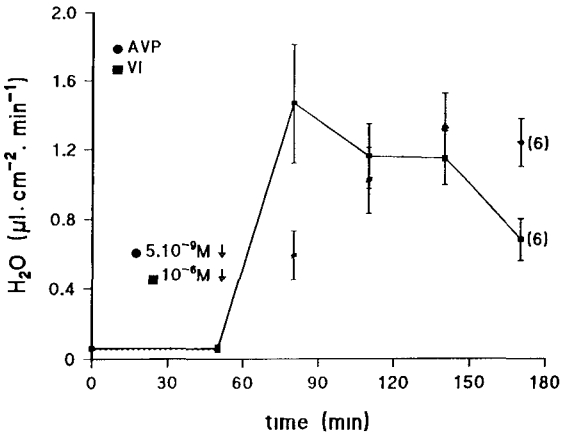

Figure 4. Comparison of the effects of AVP and analog VI on the hydroosmotic activity of frog urinary bladder cells. The Figure illustrates the effects of synthetic analogs VI – VIII. For symbols legend to see Fig. 1.

Discussion

In the first series of experiments the potency of AVP and its V_2-agonist dDAVP to stimulate transepithelial water flux in frog urinary bladder was compared. AVP is known to be a very potent stimulator of the hydroosmotic activity (Hays and Leaf 1962; Eggena et al. 1970; Mann et al. 1986), whereas for dDAVP, which is a highly potent antidiuretic agent with a high and prolonged natriferic activity (Bakoš et al. 1984; 1990b), only a low hydroosmotic activity in comparison to AVP has been previously reported (Barth et al. 1975; Bisordi et al. 1980; Mann et al. 1986). The results of the present work confirmed that dDAVP is a 40 times less potent stimulator of transepithelial osmotic water flow than AVP. Taken together, data for dDAVP indicate that while the removal of the α-amino group from the hemicystine residue at position 1 of the vasopressin molecule leads to enhanced V_2/V_1 selectivity and prolonged antidiuretic (Vávra et al. 1968) and natriferic (Bakoš et al. 1984; 1990b) activities its hydroosmotic activity is weakened.

In another series of experiments we tested the hydroosmotic activity of synthetic vasopressins obtained by modification of the vasopressin molecule in positions 1, 2 and 4. Results of this part provide *in vitro* evidence for the lack of stimulatory effects on the basal level of the frog urinary bladder wall permeability for water. On the other hand, after pretreatment of bladders with the analogs for 30 minutes and after an additional application of AVP or dDAVP the ability of these peptides to stimulate osmotic water flow was suppressed or blocked. It was shown earlier that

modifications of the AVP 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 synthetic vasopressin analogs obtained by these modifications (analogs I, II and III) show that all are potent antagonists of the vasopressor response to AVP with low antidiuretic agonistic activities which give them high antivasopressor/antidiuretic selectivity (Lammek et al. 1988). Therefore, based on our results it could be proposed that the lack of effect of these analogs on the basal transepithelial water flux is in good agreement with the concept that, in amphibian epithelia, vasopressin-sensitive water, salt and urea permeability mechanisms are accessed by a V_2-type receptor mechanism (Exton 1987; Kinter et al. 1988; Manning et al. 1988). The results also indicate that introduction of a cyclic structure at the β-carbon in position 1 (analogs I, II) and further methylation in position 2 (analog III) of the AVP molecule was associated not only with low antidiuretic (Lammek et al. 1988) and natriferic activities (Bakoš et al. 1990a) but also extremely suppressed hydroosmotic activity.

The two other synthetic analogs were obtained by modifying the parent molecule in position 4, with an additional arginine being substituted for the glutamine residue (analog IV), and the whole complex was further deaminated (analog V). Analog V had decreased antidiuretic with unchanged pressor activity; the opposite analog IV had practically negligible effect in the antidiuretic assay, whereas its pressor activity was considerably higher making this compound a pronounced pressor agonist rather than a potential pressor antagonist (Rekowski et al. 1985). Tests of natriferic activity of these synthetic analogs showed a good accordance with antidiuretic activity. Analog V had reduced natriferic activity, whereas analog IV was ineffective in this respect (Bakoš et al. 1990a). It is important to note that deamination of the new complex resulted in a reduced instead of increased antidiuretic activity. This is in discrepancy to what has been observed e.g. for dDAVP (Vávra et al. 1968). The hydroosmotic activities of these two analogs were effectively the same as those of analogs I, II and III. Stimulation of the osmotic water flow has been observed only after pretreatment of bladders with analog V and secondary application of AVP also (Table 5*d*). We can conclude that replacement of the glutamine residue in position 4 of the AVP molecule by an additional arginine results 1) in abolition of the stimulatory effect on the osmotic water flow, and 2) in inhibition or complete suppression of AVP-stimulated hydroosmotic activity of the frog urinary bladder. Secondary deamination of this complex, however, could play a role in partial preserving of hydroosmotic activity (Table 5*d*).

Another finding from these results is the ability of the analogs, which are proposed to be potent V_1-receptor antagonists, to inhibit or completely block vasopressin-stimulated osmotic water flow. In amphibian epithelia, where V_1-receptors are also present, activation of this receptor results: 1) in the breakdown

of polyphosphoinositides with the formation of inositol triphosphate and release of diacylglycerol (Ausiello et al. 1987), 2) in cellular responses that down regulate the water flow response of AVP, and 3) has been implicated as functioning in a negative feedback system on the AVP hydroosmotic response (Schlondorff and Satriano 1985; Yorio 1987). If these observations and suggestions are taken into account and if the analogs tested are selective V_1-receptor antagonists they should block AVP-stimulation but no dDAVP-induced stimulation of osmotic water flow. In experiments in which urinary bladders were pretreated with analogs I, II, III and IV no stimulation effect was observed either after additional AVP, or dDAVP (V_2-receptor agonist) application. Taken together, our results indicate that in relation to their hydroosmotic activity analogs tested could behave as mixed V_1/V_2 antagonists with increased V_1/V_2 selectivity. 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 to be not ideal agents since they preserve some intrinsic agonistic activities (Liard 1988). The reasons for these discrepancies are not obvious but they might reflect large uncontrolled variability also in the responses of the individual bladders used. Nevertheless, these results might refer to the presence of a vasopressin V_1-receptors in frog urinary bladder epithelial cells and support their need and role in realization of the hydroosmotic action of AVP.

In the last series of experiments we tested the hydroosmotic activity of three N-terminally extended [Arg^8]-vasopressins: Ala-AVP, Ser-Ala-AVP and Thr-Ser-Ala-AVP. Previous results showed that these analogs have high antidiuretic and prolonged effect *in vivo* (Lammek et al. 1987) and identical natriferic activities as compared to synthetic AVP (Ponec et al. 1990). Our data indicate that N-terminal extension of the vasopressin molecule results in abolition of its hydroosmotic activity in the range in which AVP is most potent (5.10^{-9}mol.l^{-1}). If analogs were applied in a 200 times higher concentration (10^{-6}mol.l^{-1}) the stimulatory effect on the osmotic water flow was similar to that of AVP (5.10^{-9}mol.l^{-1}). Lower concentrations were less effective or practically ineffective. Differences were observed also in time to reach maximal response. In this respect, the effects of the analogs were postponed. This delay could be explained based on the molecular mechanism of hormone-receptor binding processes and/or by a slower triggering of the specific stimulatory effect.

In summary, the testing of the hydroosmotic activity of synthetic arginine-vasopressins showed that the dDAVP:AVP concentration ratio in reaching maximal effects is 40. Modifications in positions 1, 2 and 4 of the vasopressin molecule practically totally abolished the hydroosmotic properties of the agent. After pretreatment of bladders with these analogs and after additional application of AVP, its stimulation activity was strongly suppressed or blocked. N-terminal extension of the AVP molecule did not influence the AVP capacity to induce a V_2-receptor

mediated action, but concentrations needed for reaching stimulations similar to that obtained by synthetic AVP were shifted to the a 200 times higher level.

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