Stimulation of the Sodium Transport across the Frog Skin by Three N-Terminally Extended Arginine-Vasopressins

J. PONEC[1], P. BAKOŠ[1], B. LICHARDUS[1], M. ALEXANDROVÁ[1], B. LAMMEK[2], P. REKOWSKI[2] and G. KUPRYSZEWSKI[2]

1 *Institute of Experimental Endocrinology, Slovak Academy of Sciences, Vlárska 3, 833 06 Bratislava, Czechoslovakia*
2 *Institute of Chemistry, University of Gdansk, Sobieskiego 18, 80952 Gdansk, Poland*

Abstract. The standard Ussing method was used to electrophysiologically characterize the effects of three analogs of arginine-vasopressin (AVP) on the frog skin, a model Na-transporting epithelium. The analogs tested were N-terminally extended Arg8-vasopressins: Ala-AVP, Ser-Ala-AVP and Thr-Ser-Ala-AVP; synthetic Arg8-AVP was used as the reference agent. The vasopressins were applied to the basolateral side of the frog skin in concentrations ranging between 10^{-8} to 10^{-5} mol.l^{-1}. All the three analogs increased both the short-circuit current (I_{sc}) and the open-circuit transepithelial potential (V_{oc}), and decreased the transepithelial d.c. resistance (R_t) similarly as did synthetic Arg8-AVP. The results show that N-terminal extension of the Arg8-AVP did not alter the natriferic properties of AVP.

Key words: Frog skin — Electrophysiological parameters — Arginine-vasopressin — N-terminally extended vasopressins

Introduction

In over 30 years hundreds of analogs of naturally occurring mammalian, as well as avian pituitary hormones have been synthesized and tested for their activities (Manning et al. 1987). The antidiuretic activities of vasopressin (AVP) analogs were mainly assessed in assays involving intravenous injections of the respective agents into ethanol anaesthetized, water-loaded rats (Sawyer 1958). The antidiuretic activity of AVP and its analogs (mediated via V2 receptors) in vivo rather than its pressoric activity corresponds with in vitro natriferic activity in the non-excitable epithelia of amphibian skin (Nagel 1978; Bakoš et al. 1984;

Rick et al. 1984), bladder (Rick et al. 1988) or colon (Krattenmacher et al. 1988).

Synthesis of three new N-terminally extended vasopressins was reported by Lammek et al. (1987). The three compounds — Ala-AVP, Ser-Ala-AVP, Thr-Ser-Ala-AVP — were synthesized in an effort to find analogs of AVP with protracted activity, functioning in vivo as "hormonogens", e. i. slowly releasing the parent hormone molecule by enzymatic action. Such an action was proposed and shown with Gly-Gly-Gly-vasopressin (Forsling et al. 1980). In fact, all the three molecules of N-terminally extended AVP studied here were found to have high in vivo antidiuretic activity and a prolonged effect as compared to original AVP (Lammek et al. 1987). Their vasoconstrictory activities were lower than that of AVP (Kaliszan et al. 1988).

In the present work these substances were tested in vitro for their natriferic activities, and compared to the activity of synthetic Arg8-AVP; isolated skin of the frog *Rana temporaria* was used. All the three AVP analogs were shown to possess approximately identical natriferic potencies as compared to the synthetic AVP. Consequently, N-terminal extension of Arg8-AVP seems to be without any influence on the natriferic properties of AVP as far as both the magnitude and the duration of the action are concerned.

Symbols used:
V_{oc}, open-circuit transepithelial potential ($I_{sc} = 0$)
I_{sc}, short-circuit current at zero transepithelial potential
R_t, transepithelial ohmic resistance

Materials and Methods

Animals and experimental setup. Experiments were performed within January—June on abdominal skins of male frogs *Rana temporaria*. The frogs were kept at + 5 °C until the day of the experiment. The animals were pithed and the skins were stripped and mounted between two parts of a chamber perfused with aerated recirculating frog Ringer solution (Bentley 1958). The experiments were performed at room temperature (20—22 °C). The perfusion chamber and the electronics (Nagel 1976; Higgins et al. 1977; Helman and Fisher 1977) were modified as described elsewhere (Ponec et al. 1989a, b). The skin (surface area 1 cm^2) was oriented vertically and the edges of the epithelium in contact with the parts of the perfusion chamber were embedded in high-grade silicone grease to prevent electrical leaks and to cover up the possibly damaged edges.

The electronics of the experimental layout was based on four-electrode automatic voltage clamp with high input impedance input stage. As a rule, the preparations were short-circuited throughout the whole experiment with short disconnections of the short-circuiting electronics to obtain a measure of the open-circuit transepithelial potential.

Experimental protocol. After mounting the skin in the chamber time was allowed until basal

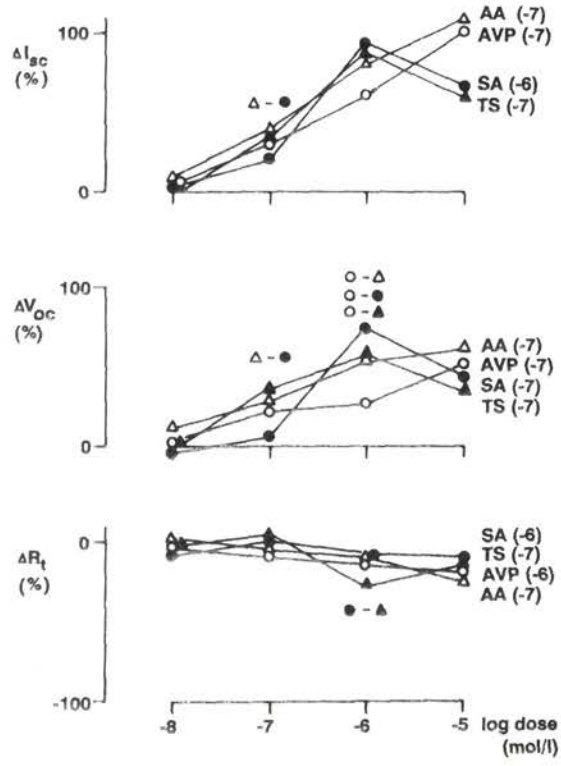

Fig. 1. Percentual changes of the short-circuit current (I_{sc}), the transepithelial open-circuit potential (V_{oc}) and the transepithelial ohmic resistance (R_t) upon addition of different concentrations of Arg8-vasopressin (AVP) and its analogues. Ala-AVP (AA); Ser-Ala-AVP (SA); and Thr-Ser-Ala-AVP (TS). The graphical symbols within response lines represent mean values calculated from 3—14 determinations. The figures in parentheses indicate the lowest concentrations (in log dose) of AVP or the analog from which the changes of the particular parameter were significantly different ($P < 0.05$) as compared to the initial levels. Clusters of graphical symbols over particular response values indicate statistically significant difference ($P < 0.05$) between the mean responses found after the application of equal concentrations of the substances. All significant differences of this kind found are shown.

parameters stabilized. Then AVP or its analogs were applied to the inner (basolateral) side of the skin without changing osmolarity, pH or volume of the bathing solution. The final concentrations of the agonists were between 10^{-8} and 10^{-5} mol $.l^{-1}$ in steps of one order of magnitude.

Calculations and statistics. The Ohm law formula was employed to calculate transepithelial d.c. resistance (Ussing and Zerahn 1951). For statistical analysis Fisher's F and Student's t-test were used. Differences of means at the $P < 0.05$ level were considered statistically significant.

Table 1. Absolute values of short-circuit current after the application of AVP and/or its analogs. Also see Fig. 1. Mean ± S.E.

Substance Control level vs Concentration (mol.l)	Arg8-AVP I_{sc} (μA.cm^{-2})	Ala-AVP I_{sc} (μA.cm^{-2})	Ser-Ala-AVP I_{sc} (μA.cm^{-2})	Thr-Ser-Ala-AVP I_{sc} (μA.cm^{-2})
Control	−37.8 ± 1.7	−35.2 ± 2.4	−55.6 ± 12.1	−25.2 ± 1.7
10^{-8}	−39.0 ± 2.1	−39.2 ± 3.3	−56.4 ± 4.1	−25.5 ± 2.0
Control	−31.1 ± 5.5	−33.6 ± 2.1	−25.9 ± 6.2	−14.4 ± 1.6
10^{-7}	−40.9 ± 5.4	−45.9 ± 2.7	−27.8 ± 5.9	−19.0 ± 1.2
Control	−35.3 ± 2.8	−27.3 ± 1.2	−27.2 ± 3.8	−26.0 ± 5.1
10^{-6}	−48.8 ± 2.4	−49.8 ± 4.3	−50.6 ± 2.1	−52.7 ± 7.3
Control	−35.5 ± 4.6	−25.2 ± 3.6	−39.1 ± 5.2	−37.0 ± 2.1
10^{-5}	−60.1 ± 4.7	−53.2 ± 6.7	−58.3 ± 4.1	−58.5 ± 3.6

Note: The negative signs reflect the fact that in the method used the short-circuit current was applied by an external voltage opposing the transepithelial potential created by the epithelium with the electrically positive basolateral side (see Materials and Methods).

Reagents. AVP agonists with N-terminally extended molecule (Ala-AVP, Ser-Ala-AVP and Thr-Ser-Ala-AVP) were synthesized at the Institute of Chemistry, University of Gdansk, Poland. The synthesis of the AVP analogs and some of their biological properties in vivo (Lammek et al. 1987) and in vitro (Kaliszan et al. 1988) were described elsewhere. Synthetic AVP was kindly provided by Dr. T. Barth, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague.

Results

The application of AVP or its agonists Ala-AVP and Thr-Ser-Ala-AVP in concentrations between 10^{-7} and 10^{-5} mol.l^{-1}, and the application of Ser-Ala-AVP in concentrations between 10^{-6} and 10^{-5} mol.l^{-1} significantly increased ($P < 0.05$, Student's t-test for paired data) the short-circuit current (Fig. 1, I_{sc}) and the transepithelial potential difference (Fig 1, V_{oc}) across the frog skin epithelium. These effects were accompanied by a decreased transepithelial d.c. resistance of the skin. As the application of all the vasopressins tested resulted in parallel changes in both the transepithelial current and potential, the transepithelial d.c. resistance changed only slightly (Fig. 1, R_t).

The dose-response relationship for AVP and the analogs Ala-AVP, Ser-Ala-AVP and Thr-Ser-Ala-AVP are also presented in Figure 1, where percentual

changes of the respective parameters (to overcome interindividual variability) have been plotted vs concentrations of the preparation. It is shown that all the hormones tested produced a nearby dose-dependent increase in the transepithelial sodium transport (reflected by the increased short-circuit current), and in the other electrophysiological parameters studied (V_{oc}, R_t).

In evaluating the data, percentual changes of the parameters studied were used instead of absolute values of the short-circuit current, open-circuit transepithelial potential, and transepithelial d.c. resistance. This approach was adopted due to interindividual variability of the absolute values, as documented in Table 1 for I_{sc}.

Discussion

The present experiments have shown that the relative potencies of N-terminally extended arginine-vasopressin analogs in stimulating the sodium transport across the frog skin are aproximatelly identical to the natriferic activity of arginine-vasopressin. The activities of all three analogs could be evaluated as high. This finding is in good agreement with the observation that the receptor induced antidiuretic (V2) activity in vivo is high (Lammek et al. 1987), this confirms the postulated match between the antidiuretic and the natriferic activities mediated through V2 receptors (Exton 1987; Manning et al. 1988).

However, some differences could be observed as to the relative potencies of the three analogs when comparing in vivo and in vitro results. In one of the in vivo studies (Lammek et al. 1987) the strongest vasopressor, but not antidiuretic activity, was induced by Ala-AVP, the analog with a natriferic potency identical to that of AVP. The antidiuretic activities of Ser-Ala-AVP and Thr-Ser-Ala-AVP in vivo were weaker in the referred study (Lammek et al. 1987) than the activity of AVP, whereas the in vitro natriferic activities of both these analogs in the present study were not different from the natriferic activity of AVP.

The irregularities in the action of the vasopressins studied on V_{oc} observed at 10^{-6} mol/l might be due to value scattering. Although R_t was calculated from both I_{sc} and V_{oc} according to Ohm's law, the mean transepithelial resistance remained insignificant due to considerable value scatter.

A protracted vasopressor and antidiuretic activities of the analogs tested were observed in vivo (Lammek et al. 1987). In the present work, no differences concerning protracted activity or a delay of maximal stimulation of the sodium transport by a particular dose were observed in vitro (not shown). This could be the result of the properties of the analogs themselves rather than that of the epithelial preparation as, in another study with the vasopressin analog dDAVP, not only a protracted activity of the analog but also a delayed onset of maximal

stimulation of the natriferic responses have been observed (Bakoš et al. 1984).

Ser-Ala-AVP and Thr-Ser-Ala-AVP in concentrations of 10^{-5} mol/l tend to show a less pronounced activity (the means compared to the the mean values obtained at 10^{-6} mol/l being insignificant). This might be seen as deteriorating the dose-response linearity as compared to the action of AA and AVP. It is not possible to estimate on the basis of the present results whether a N-terminal extension of the AVP molecule by 2 and 3 amino acids could be responsible for this difference, and whether this phenomenon could be linked to different diffusion of the longer (SA, TS) vs shorter molecules (AA, AVP) to the sites of action of the hormone.

In summary, the testing of the natriferic activities of the three Arg8-AVP analogs has shown that N-terminal extension of the AVP molecule did not influence the AVP capacity to induce a V2 receptor mediated action.

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