Comparison of the Effects of dDAVP and AVP on the Sodium Transport in the Frog Skin

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Abstract. The effect of 1-deamino-8-D-arginine-vasopressin, dDAVP, the synthetic analogue of vasopressin, upon the active sodium transport across the frog skin was studied using standard microelectrode technique and compared with the effect of synthetic arginine-vasopressin, AVP. dDAVP applied to the basolateral side of the epithelium stimulated the active sodium transport as reflected by the increase of short-circuit current, I_{sc}, and transepithelial electrical potential difference, V_{oc}. Potential difference across both the apical, V_0, and the basolateral, V_i, cell membranes decreased. The driving force of transepithelial sodium transport, E_{Na}, did not change. The transepithelial electrical resistance, R_t, ohmic resistance of the active sodium transport, R_{Na}, and apical cell membrane resistance, R_0, rapidly decreased, while the resistance of the basolateral cell membrane, R_i, and the resistance of the shunt pathway, R_s, remained unchanged. It is concluded that dDAVP primarily increases sodium permeability of the apical cell membrane which subsequently stimulates sodium pump activity. This action is similar to that of AVP.

Key words: Frog skin — Cell membranes — Microelectrode study — dDAVP — AVP

Introduction

The mechanisms of hormonal action on membrane level have been intensively studied using microelectrode techniques (Helman et al. 1975; Nagel 1976; Helman and Fisher 1977; Nagel 1978; Els and Helman 1981; Schoen and Erlij 1985a; Schoen et al. 1988; Krattenmacher and Clauss 1988). There is compelling evidence that the apical membrane sodium permeability of the cells of tight epithelia, such as frog skin, is controlled mainly by neurohypophyseal hormones that alter intracellular concentration of cyclic 3′,5′-adenosine monophosphate, cAMP. This has been well documented for vasopressin (Handler and Orloff 1973; Andreoli and Schafer 1976). Arginine-vasopressin apparently increases the permeability of the amiloride-sensitive Na^+ pathway of the apical cell

membrane (MacKnight et al. 1980; Helman et al. 1981; Li et al. 1982) whereby the intracellular sodium concentration is increased (Rick et al. 1984; 1988) and this in turn stimulates the sodium pump in the basolateral membrane (Rick et al. 1984). Electrophysiologically, the increase of apical membrane permeability in the isolated frog skin manifested itself as an increase in apical membrane slope conductance with little or no change of the electrophysiological parameters of the basolateral membrane (Nagel 1978; Els and Helman 1981). However, neurohypophyseal hormones have recently been found to produce an increase in the basolateral membrane conductance in addition to the effect on the apical membrane (Schoen and Erlij 1985b, c; Erlij et al. 1986; Van Driessche and Erlij 1988; Schoen et al. 1988).

The synthetic analogue of vasopressin, 1-deamino-8-D-arginine-vasopressin, has a protracted antidiuretic activity while having practically no pressoric effect when applied in vivo in vertebrates (Zaoral et al. 1967). It also stimulated sodium transport through the frog skin with the effect being more protracted as compared to that of arginine-vasopressin or lysine-vasopressin (Bakoš et al. 1984). However, no electrophysiological characterization of dDAVP action on cell membrane level of tight Na^+ transporting epithelium has been performed as yet.

The present work was aimed at studying the action of dDAVP on sodium transport across the frog skin cell membranes in comparison to that of AVP by using standard microelectrode technique. It was shown that AVP and its synthetic analogue dDAVP stimulate active sodium transport across the frog skin in a similar way and by similar mechanisms.

V_t, transepithelial potential difference
V_{oc}, open-circuit transepithelial potential (V_t at $I_{sc} = 0$)
I_{sc}, short-circuit current at zero transepithelial potential ($V_t = 0$)
V_o, potential across the apical (outer) cell membrane at $V_t = 0$
V_i, potential across the basolaterall (inner) cell membrane at $V_o = 0$ ($V_i = V_t$ at $V_o = 0$)
E_{Na}, driving force of the transepithelial sodium transport
R_s, ohmic resistance of the shunt pathways
R_t, transepithelial ohmic resistance
R_{Na}, ohmic resistance of the active sodium transport pathway
R_o, ohmic resistance of the apical (outer) cell membrane
R_i, ohmic resistance of the basolateral (inner) cell membrane
Fr_o, fraction resistance of the apical (outer) cell membrane expressed as percentage of R_{Na} ($R_{Na} = R_o + R_i$)

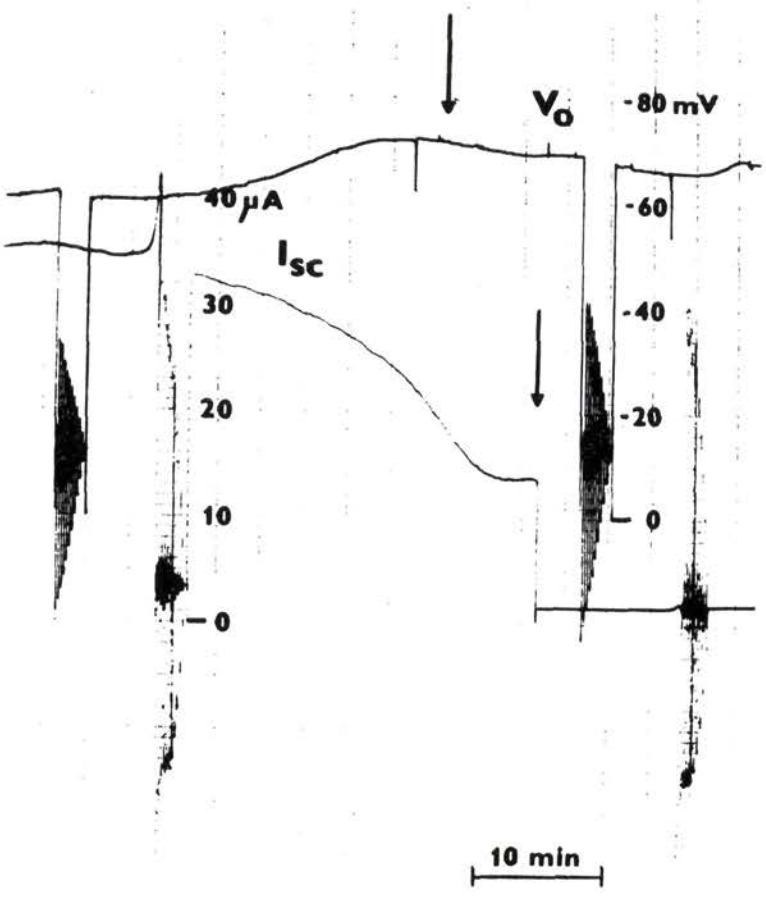

Fig. 1. Representative recording of the short-circuit current, I_{sc}, and the potential difference across the apical cell membrane, V_o, after the application of vasopressin. Arrows indicate the addition of the hormone to the solution at the basolateral skin surface. Responses of the epithelium to external transepithelial pulses of changing amplitude (from −180 to +200 mV) in 20 mV steps were measured during the control period and after reaching the maximal hormonal effect.

Materials and Methods

Animals and experimental setup: Experiments were performed on skins of European frogs (*Rana temporaria*) of both sexes throughout the year. The frogs were kept at +5 °C until the day of experiment when they were pithed, the abdominal skin stripped and mounted horizontally between two halves of a Ussing-type chamber (area = 1 cm^2) perfused with recirculating Ringer solution (Bentley 1958). The volume of the upper and the lower halfchamber was 15 and 10 ml, respectively. The experiments were performed at room temperature (20—22 °C). Skin orientation in the chamber

Table 1. Effect of dDAVP on absolute values of I_{sc}, V_{oc}, V_o, V_i, E_{Na}, R_s, R_t, R_{Na}, R_o, R_i, R_o/R_i, fR_o. For symbols see Introduction. Values are means ± SEM (ranges are shown). Number of experiments $n = 14$.

	Control (Range)	dDAVP (Range)	Significance level
I_{sc} [μA . cm^{-2}]	−34.2 ± 4.8 (−62.2 to −13.8)	−54.2 ± 7.1 (−108.8 to −20.3)	$P < 0.001$
V_{oc} [mV]	50.1 ± 6.4 (20.7 to 104.4)	61.0 ± 4.8 (31.3 to 88.8)	$P < 0.005$
V_o [mV]	−62.2 ± 3.7 (−88.7 to −40.8)	−39.8 ± 3.7 (−70.6 to −19.2)	$P < 0.001$
V_i [mV]	96.4 ± 3.4 (78.7 to 129.5)	86.7 ± 4.7 (60.9 to 123.7)	$P < 0.02$
E_{Na} [mV]	137.8 ± 5.5 (112.4 to 163.8)	138.1 ± 6.0 (106.3 to 180.4)	n. s.
R_s [ohm . cm^2]	2805 ± 365 (800 to 6075)	2473 ± 264 (1501 to 4360)	n. s.
R_t [ohm . cm^2]	1623 ± 148 (627 to 2533)	1266 ± 102 (763 to 1808)	$P < 0.005$
R_{Na} [ohm . cm^2]	5463 ± 871 1929 to 11 244)	3309 ± 523 (1415 to 7046)	$P < 0.001$
R_o [ohm . cm^2]	3654 ± 648 (885 to 7874)	1589 ± 320 (431 to 4617)	$P < 0.001$
R_i [ohm . cm^2]	1809 ± 314 (431 to 4517)	1720 ± 296 (701 to 4371)	n. s.
R_o/R_i	2.32 ± 0.44 (0.76 to 7.06)	1.03 ± 0.21 (0.34 to 3.48)	$P < 0.001$
fR_o [%]	64.8 ± 3.4 (43.1 to 87.6)	46.1 ± 3.8 (25.6 to 77.7)	$P < 0.001$

allows microelectrode impalement from the apical surface. The method used to mount the tissue in the chamber and the computer-driven voltage clamp circuit have been described in detail previously (Ponec et al. 1989a, b). To prevent electrical leaks and to cover up the possibly damaged edges, the space between the skin and the hemichambers was sealed by a thin layer of high-grade silicone grase.

Intracellular potential (potential across the apical cell membrane), V_o, was measured with standard glass microelectrodes with a tip diameter of approximately 1 μm, prepared from borosilicate glass tubes with two inner filaments (Glass Factory, Bratislava, Czechoslovakia). The electrodes were prepared using a vertical puller (Ukrainian Academy of Sciences, Kiev, USSR) and filled with 3 mol . l^{-1} KCl. The impalements of the skin were done using a mechanical micromanipulator (Leitz,

Table 2. Effect of AVP on absolute values of I_{sc}, V_{oc}, V_o, V_i, E_{Na}, R_s, R_t, R_{Na}, R_o, R_i, R_o/R_i, fR_o. For symbols see Introduction. Values are means ± SEM (ranges are shown). Number of experiments $n = 7$.

	Control (Range)	dDAVP (Range)	Significance level
I_{sc} [μA . cm^{-2}]	−31.3 ± 3.4 (−44.2 to −18.8)	−50.6 ± 6.0 (−75.6 to −32.0)	$P < 0.005$
V_{oc} [mV]	44.6 ± 4.5 (25.6 to 60.6)	55.6 ± 4.5 (42.8 to 70.8)	$P < 0.01$
V_o [mV]	−51.6 ± 3.7 (−69.5 to −42.7)	−32.6 ± 2.7 (−47.5 to −24.9)	$P < 0.001$
V_i [mV]	78.7 ± 5.3 (63.3 to 105.9)	75.6 ± 6.6 (58.3 to 108.0)	n. s.
E_{Na} [mV]	148.6 ± 6.4 (116.4 to 166.7)	150.0 ± 7.9 (116.2 to 172.4)	n. s.
R_s [ohm . cm^2]	2144 ± 261 (1375 to 3522)	1966 ± 299 (1242 to 3683)	n. s.
R_t [ohm . cm^2]	1455 ± 120 (627 to 2533)	1149 ± 92 (763 to 1808)	$P < 0.005$
R_{Na} [ohm . cm^2]	5124 ± 689 (3225 to 8866)	3286 ± 491 (1894 to 4978)	$P < 0.05$
R_o [ohm . cm^2]	3318 ± 401 (2365 to 5550)	1438 ± 237 (755 to 2464)	$P < 0.002$
R_i [ohm . cm^2]	1806 ± 322 (431 to 4517)	1848 ± 305 (701 to 4371)	n. s.
R_o/R_i	2.27 ± 0.59 (1.30 to 5.74)	0.82 ± 0.10 (0.48 to 1.23)	$P < 0.05$
fR_o [%]	65.9 ± 3.5 (43.1 to 87.6)	44.0 ± 3.3 (25.6 to 77.7)	$P < 0.001$

FRG). The criteria for acceptable punctures were in accordance with those described by other investigators (Tang et al. 1985; Schoen and Erlij 1985a).

To determine the current-voltage relations of the skin, the transepithelial potential difference, V_t, was changed by applying a series of transepithelial pulses of different amplitudes (−180 to +200 mV) in 20 mV steps (Fig. 1). The duration of the pulses until data sampling was 600 ms to avoid polarization effects on the basolateral membrane at the onset of the pulse (Schoen and Erlij 1985b, 1987). Standard formulae were employed for data evaluation and for the calculation of electrophysiological characteristics of the epithelium from current-voltage relationships (Ussing and Zerahn 1951; Helman et al. 1975; Issacson 1977; Nagel 1978). Students's *t*-test of paired data was used for statistical analysis.

Table 3. Comparison of the effects of dDAVP and AVP on I_{sc}, V_{oc}, V_o, V_i, E_{Na}, R_s, R_t, R_{Na}, R_o, R_i, R_o/R_i, fR_o expressed as percentage differences. For symbols see Introduction. Values are means ± SEM (ranges are shown). Number of experiments (dDAVP: $n = 14$; AVP: $n = 7$).

	dDAVP (Range)	AVP (Range)	Significance level
I_{sc} [μA . cm^{-2}]	65.9 ± 10.0 (16.9 to 161.4)	64.6 ± 15.6 (13.4 to 140.4)	n. s.
V_{oc} [mV]	32.3 ± 8.1 (−19.8 to 91.3)	28.8 ± 10.9 (8.0 to 92.6)	n. s.
V_o [mV]	−36.6 ± 4.0 (−59.6 to −5.9)	−36.5 ± 2.7 (−46.5 to −26.4)	n. s.
V_i [mV]	−10.0 ± 3.8 (−30.9 to 22.6)	−4.5 ± 3.0 (−10.1 to 10.6)	n. s.
E_{Na} [mV]	0.73 ± 3.1 (−17.5 to 20.4)	1.8 ± 6.3 (−18.7 to 34.5)	n. s.
R_s [ohm . cm^2]	−0.30 ± 10.9 (−72.3 to 110.3)	−8.7 ± 3.5 (−18.8 to 4.6)	n. s.
R_t [ohm . cm^2]	−17.8 ± 5.7 (−60.6 to 34.3)	−20.6 ± 3.5 (−32.5 to −4.5)	n. .s
R_{Na} [ohm . cm^2]	−37.4 ± 2.9 (−55.1 to −20.4)	−34.6 ± 7.8 (−59.6 to −2.9)	n. s.
R_o [ohm . cm^2]	−55.0 ± 3.9 (−75.0 to −24.7)	−56.8 ± 5.5 (−68.0 to −27.5)	n. s.
R_i [ohm . cm^2]	1.8 ± 9.0 (−34.1 to 76.8)	15.5 ± 18.4 (−43.7 to 90.7)	n. s.
R_o/R_i	−51.4 ± 6.0 (−78.4 to −2.4)	−59.3 ± 4.8 (−78.6 to −37.7)	$P < 0.05$
fR_o [%]	−28.9 ± 4.5 (−56.9 to −1.5)	−33.2 ± 3.4 (−43.0 to −18.4)	$P < 0.01$

Experimental protocol: After mounting the isolated skin in the perfused chamber time was allowed until basal parameters stabilized. Then several control impalements and measurements of the skin transport parameters were done. Synthetic analogue of vasopressin, 1-deamino-8-D-arginine-vasopressin (obtained from the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague; in some experiments Adiuretin SPOFA, dDAVP solution with identical characteristics), was added to the solution bathing the basolateral frog skin surface. The final concentration of dDAVP was 10^{-5} mol . l^{-1} ($n = 14$ experiments).

In another series of experiments synthetic arginine-vasopressin, (Calbiochem), was used ($n = 7$ experiments). The experimental setup and the concentrations used were the same as those during dDAVP application. The cells were impaled and current-voltage relationships were estimated predominantly during the phase of maximal effects of the hormones. The concentrations of both

hormones were chosen so as to obtain maximal stimulation of the sodium transport across the frog skin expressed as the change of the short-circuit current.

Results

The effect of dDAVP

The application of dDAVP to the basolateral side of the frog skin highly significantly stimulated the short-circuit current, I_{sc}, and the transepithelial potential difference, V_{oc}, measured under open-circuit conditions. The potential difference across the apical cell membrane, V_o, as well as potential across the basolateral cell membrane, V_i, decreased. The electromotoric driving force of transepithelial sodium transport, E_{Na}, did not change after the addition of dDAVP (Table 1).

The transepithelial ohmic resistance of the skin, R_t, and the resistance of the active sodium transport pathway, R_{Na}, decreased. This change was proportional to the increase of the short-circuit current, thus providing a measure of the level of the frog skin active sodium transport. A significant decrease of the resistance of the apical cell membrane, R_o, was observed, whereas the resistance of the basolateral cell membrane, R_i, remained unchanged. dDAVP also decreased the cell membrane resistances ratio, R_o/R_i, and the percentual share of the apical membrane, fR_o. The resistance of the shunt transport pathway, R_s, did not change after dDAVP application (Table 1).

The effect of AVP

Similar results were obtained with the application of equimolar doses of AVP (Table 2). Short-circuit current, I_{sc}, and transepithelial potential difference, V_{oc}, increased. The potential across the apical cell membrane, V_o, decreased, however, the level of the potential difference across the basolateral cell membrane, V_i, remained unchanged. The driving force of transepithelial sodium transport, E_{Na}, did not change during AVP application, similarly as it was the case with dDAVP.

The AVP-induced changes of the ohmic resistance were the same as those after dDAVP treatment (Tables 1, 2).

Comparison of the effects of dDAVP and AVP

Table 3 compares the effects of dDAVP and AVP, expressed as percentual differences. It is evident from Table 3 that both hormones influenced frog skin

sodium transport parameters in a similar manner. Significant differences concerned only the cell membranes resistance ratio, R_o/R_i, and the fractional resistance of the apical cell membrane, fR_o.

Discussion

The present experiments showed that the natriferic response of the frog skin epithelium to the synthetic analogue of arginine-vasopressin, 1-deamino-8-D-arginine-vasopressin differred little, if at all, from that of the AVP effect. Both dDAVP and AVP markedly stimulated active sodium transport across the skin as expressed in values of short-circuit current, I_{sc}, (dDAVP: +65.9 %; AVP: +64.6 %; Table 3). The decrease of the potential across the apical cell membrane, V_o, was also similar (dDAVP: −36.6 %; AVP: −36.5 %). Changes of basolateral membrane potential expressed as percentages, were insignificant. These two findings agree well with the changes of both the apical and the basolateral ohmic resistances, R_o and R_i. The unchanged basolateral ohmic resistance, R_i, is in accordance with the results obtained by Els and Helman (1981) but contrasts with those of Nagel 1978, who found an increase in this parameter after AVP application. The reasons for these discrepancies are not obvious but they might reflect large uncontrolled variability in the responses of the individual skins used. This variability is probably due to different genetic lines of the animals (Rick et al. 1984).

The electromotoric driving force of transepithelial sodium transport, E_{Na}, was also unaffected by addition of dDAVP and/or AVP. This finding is in accordance with other reports (Yonath and Civan 1971; Nagel 1978; Els and Helman 1981; Lau et al. 1981). However, also contradictory results have been published: both increase in E_{Na} (Andreoli and Schafer 1976; Lang et al. 1977; Issacson 1977; Rick et al. 1984) as well as decrease in this parameter (Hong and Essig 1976; Concha et al. 1987). These discrepancies may be accounted for by differences in the methodology of measuring or calculating this important transport parameter. Estimation of E_{Na} might be different as E_{Na} is a complex parameter, which depends on energetic and kinetic (permeability) factors of the transport systems. The changes of this parameter under hormonal influence is thus open for discussion. Our data provide evident proportional changes in short-circuit current, I_{sc}, and tissue resistance, R_t, (Table 3), which might yield unchanged E_{Na}.

Both dDAVP, and AVP similarly decreased the transepithelial tissue resistance, R_t, resistance of the active sodium transport pathway, R_{Na}, and also the resistance of the apical cell membrane, R_o, while the resistance of the basolateral

cell membrane, R_i, and the resistance of the shunt pathways, R_s, remained unchanged (Tables 1, 2, 3). It is known that the shunt pathway resistance is basically represented by resistances of both the paracellular junctions and glandular cells (Lindemann and Voûte 1976). It was also shown that neurohypophyseal hormones increase the permeability of amiloride-sensitive, voltage-dependent sodium channels in tight epithelia (Helman et al. 1981; Li et al. 1982; Schoen et al. 1988). Amiloride selectively blocks the cellular current pathway without modifying any parallel pathways (Nielsen 1982; Fisher and Lockard 1988). It can thus be proposed that dDAVP, like AVP, primarily increases apical sodium permeability (as shown in this work by the decreased apical membrane potential, V_o, ohmic resistance, R_o, and its percentual share on the total transcellular resistance, fR_o). This conclusion is also supported by the decreased resistance ratio of the apical and the basolateral cell membranes, R_o/R_i, induced by the action of the hormones studied (Tables 1, 2, 3). Significant differences found between the last two transport parameters, either after dDAVP or AVP application, could be explained by the wide ranges of the data obtained.

It is concluded that the synthetic analogue of vasopressin, dDAVP, has a similar mechanism of action upon frog skin sodium transport as does arginine vasopressin, AVP. It is assumed that dDAVP, like AVP, acts primarily on the apical cell membrane sodium permeability, which increased after hormone application. The intracellular concentration of sodium also increased. This process is the result of the hormone action on the density of activated sodium channels localized in the apical membranes of the epithelial cells (Li et al. 1982). Increased intracellular sodium concentration then secondarily stimulates the transport enzyme, Na-K-ATPase, activity in the basolateral membrane, which was well documented by the increased short-circuit current, a measure of actively transported sodium ions from the cells into the intercellular space.

Acknowledgement. The authors wish to thank Dr. T. Barth, DrSc. for the kind supply of the synthetic analogue of vasopressin.

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Final version accepted September 11, 1989