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The effect of vasopressin on the permeability of frog skin to cations

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SUMMARY

The effect of vasopressin on the permeability of isolated frog skin to some cations was studied by determining the permeability coefficients of K^+, Cs^+ and Rb^+ in relation to that of Na^+, with and without supramaximal doses of vasopressin. The relative permeability coefficients for Cs^+ and Rb^+ are much lower in the presence than in the absence of vasopressin, while for K^+ it remains unaltered.

Changes in Na^+ concentration in the solution bathing the outside face of isolated frog skin produce alterations in the open-circuit potential which have been interpreted as resulting from a diffusion potential created at some point near the outside face[1], implying the existence of a barrier selectively permeable to Na^+. It is widely accepted that antidiuretic hormone increases the short-circuit current and the potential across the frog skin by increasing Na^+ transport[2]. The assumption is made that this effect of antidiuretic hormone results from an increase in the permeability of the outside barrier to Na^+. Andersen and Ussing[3] have suggested that this barrier is composed of two barriers in series, an outer barrier which is permeable to water, Na^+ and to small non-polar molecules and much less permeable to other polar molecules, and a second barrier, non-selective, with water-filled channels, which is the rate limiting point for Na^+ diffusion. According to Leaf and Hays[4], antidiuretic hormone is effective on this second barrier; this model explains why the hormone increases the mucosal to serosal fluxes of Na^+, water and small, non-polar molecules across the toad bladder. We could thus predict that antidiuretic hormone would increase the permeability coefficient of Na^+ in relation to that of other cations.

Lindley and Hoshiko[5] studied the changes in the potential difference across frog skin caused by changing the cation species and concentrations in the outside solutions and determined the permeability coefficients of different cations in relation to Na^+, using

the Goldman–Hodgkin–Katz constant field equation[6]. The following series of experiments were designed to study how these coefficients for K^+, Rb^+ and Cs^+ would be affected by the presence of antidiuretic hormone.

Frogs of the species *Rana ridibunda* Pallas were used. Pieces of abdominal skin were mounted in Ussing-type chambers with 3.14 cm^2 cross-sectional area. The composition of the solutions used is given in Table I. The osmolality was 0.150 for the reference solution and 0.03 for all the other solutions used to bathe the outside face of the skin. They were buffered with tris(hydroxymethyl)aminomethane to pH 8 after equilibration with air. The overall potential difference (P.D.) and the short circuit current (s.c.c.) were monitored by means of a Keithley 610B electrometer and a Universal Avometer. The skins were mounted with the same solution on both sides (reference Solution A) and a period of about 30 min was allowed for a stable potential to be reached. Successive replacements of the outside solutions followed, according to the protocol described below. Measurements of P.D. and s.c.c. were made every 10 min approximately, so that values were obtained just before and after each replacement and at least once more in the mean time. The reference solution in the outside chamber was first substituted by the control Solution B (20 mM Na_2SO_4) and a new, stable control P.D. was reached within 20–30 min. This solution was then substituted by the experimental solutions in which Na^+ concentration was successively halved by substitution with K^+, Rb^+ or Cs^+. This was done by mixing equal volumes of a solution with a given Na^+ concentration and one of the Solutions C (see Table I) which had 20 mequiv/l of the cation used in that particular experiment. Each experimental period lasted for 20–30 min and was followed by an identical control period (control solution outside) so that the basal conditions could be evaluated. The smallest sodium concentration used was 1/32 that of the control solution. In most experiments the order of the replacements was from the highest to the lowest Na^+ concentration, but this was inverted in some cases without any appreciable change in the results.

In a second series of experiments the same protocol was repeated in the presence of vasopressin (Pitressin Parke Davis). A reference solution containing vasopressin in the concentration of 200 I.U./l was introduced in the inside chamber at the beginning of the first control period. This was extended for about 1 h to allow for the full drug effect to

TABLE I

COMPOSITION OF THE SOLUTIONS USED IN THE EXPERIMENTS

Values expressed in mequiv/l for the electrolytes and in mM for Tris.

		SO_4^{2-}	Na^+	K^+	Cs^+	Rb^+	*Tris*
Reference Solution	A	60	115	5	–	–	0.6
Control Solution	B	10	20	–	–	–	0.6
Experimental Solution	C_1	10	–	20	–	–	0.6
Experimental Solution	C_2	10	–	–	20	–	0.6
Experimental Solution	C_3	10	–	–	–	20	0.6

be reached, and then the outside solution replacements were made as in the first group. The control value of the P.D. after the addition of vasopressin was approx. 160% that of the initial value.

In almost every case a steady downwards drift of the control potential was recorded during the time of the experiments. Therefore the values of ΔV given in Table II were determined by a graphic method, by drawing a base line across the line of the control potentials and using it as a "zero" reference. We rejected every skin in which the first control potential was smaller than 65 mV, and those in which the control potential decreased markedly; for this reason we are not considering Na^+–Li^+ replacements as we were never able to record a reasonably steady state when we tried to use this cation. In the Cs^+ experiments it was often necessary to lengthen the control periods to obtain a good recovery of the potential.

TABLE II

VALUES OF ΔV (CONTROL P.D. – EXPERIMENTAL P.D.) IN mV AT DIFFERENT OUTSIDE Na^+ CONCENTRATIONS WITH AND WITHOUT VASOPRESSIN

The total cation concentration in the outside solution was always 20 mM, Na^+ being substituted by K^+, Cs^+ or Rb^+. Means and S.D.; number of experiments in parentheses. n.s., not significant.

Substitute cation	$[Na^+]_{exp}/[Na^+]_{control}$				
	1/2	*1/4*	*1/8*	*1/16*	*1/32*
K^+	10.4	20.6	31.9	42.6	50.8
(20)	±2.3	±4.7	±6.8	±7.8	±8.3
K^+ + vasopressin	12.0	21.2	31.7	42.1	51.5
(24)	±1.7	±2.8	±3.1	±4.1	±3.9
P (*t* test)	< 0.02	n.s.	n.s.	n.s.	n.s.
Cs^+	11.1	19.3	30.8	39.5	48.7
(8)	±1.0	±3.0	±3.4	±5.2	±2.1
Cs^+ + vasopressin	14.7	26.1	36.7	49.0	61.6
(9)	±2.5	±4.1	±4.7	±4.6	±3.2
P (*t* test)	<0.01	<0.01	<0.01	<0.01	<0.01
Rb^+	10.4	19.0	28.3	37.8	
(16)	±1.7	±1.7	±3.2	±4.8	
Rb^+ + vasopressin	10.6	21.5	32.5	43.1	54.2
(8)	±2.2	±2.9	±3.2	±2.0	±2.6
P (*t* test)	n.s.	<0.02	<0.01	<0.01	

The results of these experiments, summarized in Table II, show a stepwise decrease of the P.D. related to the decrease in Na^+ concentration and dependent on the relative permeability of the substitute cation and on the presence of vasopressin. Student's *t* test was applied to verify whether the differences between the P.D. values without or

TABLE III

COMPUTED PARAMETERS OF THE EISENMAN AND GOLDMAN EQUATIONS FOR THE DIFFERENT SUBSTITUTES OF Na^+, WITH AND WITHOUT VASOPRESSIN

	Eisenman equation		*Goldman equation*
			α
	n	K_{pot}	
K^+	1.82	0.050	0.140
K^+ + vasopressin	1.59	0.069	0.138
Cs^+	1.57	0.078	0.159
Cs^+ + vasopressin	1.64	0.031	0.082
Rb^+	1.45	0.132	0.216
Rb^+ + vasopressin	1.95	0.036	0.126

with the hormone were statistically significant; the respective P values are also given in Table II.

We computed our data in order to determine the permeability coefficients of the different cations in relation to Na^+ with and without the presence of vasopressin, using the modified Goldman–Hodgkin–Katz equation (1) and the Eisenman equation (2) (ref. 7)★.

The best fit was obtained by applying Eisenman's formula, but the results are identical, qualitatively, with either equation. The permeability coefficients for Cs^+ and Rb^+ in relation to Na^+ decrease significantly in the presence of vasopressin, and the coefficient for K^+ remains unaltered. The results obtained with Cs^+ and Rb^+ are in agreement with the model of Leaf and Hays[4]. The invariance of the relative permeability coefficient of K^+ after adding antidiuretic hormone may be interpreted assuming that there is an increase of intracellular K^+ in the presence of vasopressin, due to the enhancement of the active transport of Na^+ at the inner barrier.

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★ (1) $\Delta V = (RT/F) \ln [Na_2/Na_1 (1-\alpha) + \alpha]$
(2) $\Delta V = (nRT/F) \ln \{ 1/[(Na_2/Na_1)^{1/n} + (K_{pot} (1-Na_2/Na_1))^{1/n}] \}$
where Na_2/Na_1 is the ratio of Na^+ concentrations between experimental and control conditions, α and K_{pot} are the permeability coefficients of each cation in relation to Na^+ and n is a parameter dependent on the characteristics of the membrane and of the pair of cations under study.