CONDITIONING FACTORS THAT INFLUENCE THE SPONTANEOUS VOLTAGE GRADIENT AND THE TRANSEPITHELIAL RESISTENCE IN DIVERSE IN VITRO MAMMALIANS AND AMPHIBIANS EPITHELIA

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ABSTRACT

An experimental study about spontaneous voltage gradient, total transepithelial resistance and short-circuit current is made on different amphibian and mammalian epithelia. The dissected tissue is placed between two cubic chambers. The biological membrane divides two electrolytic oxigenated-solutions at 20°C and pH 7.4 conditions. A multimeter measures the voltage difference between the solutions, then 6μA of direct current is applied through them for transepithelial resistance calculation. Thereafter a 9 – 56 V outer electromotive force and a potential divider are placed in series with the tissue to adjust and maintain zero potential, in order to measure the short-circuit current. Significant differences with 0.01level confidence were found in the electric epithelial parameters of the frog skin and urinary bladder and in the rabbit and rat proximal colon, urinary bladder and gallbladder. While [Na^+] decrease from 107 to 0.375 mM minimized the short-circuit current in the frog skin, 100 mU/ml vasopressin in the serosal solution significantly increased it by 62%. The frog urinary bladder transepithelial resistance increased by means of pH decrease from 7.4 to 6.0 in the serosal solution and also by 10 mM amiloride in the mucosal solution. Neither the total transepithelial resistance nor the shor-circuit current significantly changed in the rabbit urinary bladder exposed to amiloride.

KEYWORDS

RESUMEN

Se realiza un estudio experimental sobre el gradiente de voltaje espontáneo, la resistencia transepitelial total y la corriente cortocircuito en algunos epitelios de anfibios y mamíferos. Se coloca el tejido disecado entre dos hemicámaras cúbicas de acrilato. El tejido separa a dos soluciones electrolíticas oxigenadas, a 20°C y pH 7.4. Un multímetro mide la diferencia de voltaje entre las soluciones y luego se inyecta 6μA de corriente para calcular la resistencia transepitelial. Luego se coloca una fuerza electromotriz externa de 9 – 56 V y un resistor variable en serie con el tejido para medir la corriente cortocircuito cuando la diferencia de voltaje es 0 mV. Se encontraron diferencias significativas en los parámetros eléctricos transepiteliales medidos en la piel y vejiga urinaria del sapo, en la vesícula biliar, colon y vejiga urinaria del conejo y de la rata, con un nivel de confianza de 0.01. La disminución de la [Na$^+$] de 107 a 0.375 mM disminuyó la corriente cortocircuito en la piel de sapo. La administración de 100 mU/ml de vasopresina en el lado seroso de la piel de sapo aumentó significativamente la corriente en un 62%. La resistencia transepitelial aumentó por efecto de la disminución del pH de 7.4 a 6.0 en la vejiga urinaria del sapo y también cuando se administró 10 mM amilorida en su lado mucoso. Ni la resistencia ni la corriente cortocircuito variaron significativamente en la vejiga urinaria de conejo expuesta a amilorida.

PALABRAS CLAVES

Resistencia transepitelial, voltaje espontáneo, transporte iónico, cámara oxigenada.

INTRODUCTION

Epithelial cell membranes contain different types of ion channels that determine the total current of the cell membrane. It is possible to distinguish currents derived from passive and active ionic transports. Ussing & Zerahn (1951, republished in 1999) invented an 80 ml cubic chamber that has his surname and probably never imagined the wide range of applications this system would have. Today there are spheroideal microchambers (Pedersen et al., 1999; Negrete et al., 1996) with 0.3 ml at least (Carrasquer et al., 1999; Veeze et al., 1991) . An oxygenated two-baths system hermetically divided by the biological membrane is used in the actual research. The electrophysiological characteristics of the in vitro epithelia from the frog skin and urinary bladder, from the rabbit colon, urinary bladder and gallbladder and from the rat colon and urinary bladder are compared in an opened then closed electric circuit. Low sodium concentration, changes in the pH solutions, amiloride and vasopressin manipulations in the solutions are also evaluated. Epithelial cells form a tridimensional syncytium that functions as a unique cell with assymetric ionic transports in the apical

with respect to the basolateral membrane, in order to generate polarity or potential gradient. It is convenient to consider the epithelium as an equivalent circuit where an apical membrane resistance is in series with the basolateral membrane resistance. These membrane resistances are in parallel with the tight junction resistance, which are specially high resistences in cultured tissues (Handler et al., 1981). According to Kirchoff Law, total transepithelial resistance depends on the three resistances mentioned above (Hug 2002). The short-circuit current is due to ionic active transport, principally the sodium flow. There is an epithelial sodium channel in the apical membrane and the Na^+-K^+ ATPase pump in the basolateral one, which permit the first a net sodium movement into the cell and the second the exit from it. Traditionally, short-circuit current (Isc) is defined as a direct macroscopic current that opperates in the epithelium when the cell membrane voltage with identical Ringer solution at both sides of the tissue is clamped to zero potential. The short-circuit current is the sum of the currents derived from individual ionic channel populations that fluctuate between an opened and a closed state, just to induce very low current variations (Baker et al., 2002). Some conditions and reagents that decrease Isc are: low apical sodium charge, amiloride (Mauro et al., 1987), ouabain (Beltowski 2003), low oxygen tension, tetrodotoxin (Li et al., 1994) and low temperature. Other conditions increase the current: vasopressin, forskolin, aldosterone (Grubb et al., 1987), $CaCO_3$ crystall accumulations in the urinary bladder (Loo et al., 1985), PGE_2, PGI_2 (Pohlman et al., 1983) and teophyllin (Knauf et al., 1984). Less appreciated is that these epithelial functions can be modified by macromolecules other than neurotransmitters and hormones (Lewis et al., 1995). The general objective of the present study is to measure the electrical transepithelial parameters in amphibians and mammalians by using an in vitro model system, based on the Ussing Chamber, with specific modifications in its construction. The specific objectives are the following: 1-Compare the spontaneous voltage gradient, the transepithelial resistance and the short circuit current between the frog and the rabbit urinary bladders; between the rabbit urinary bladder and gallbladder; between the frog skin and urinary bladders; between the rabbit and the rat urinary bladders and colons and between the rabbit urinary bladder and colon. 2-Observe the low sodium concentration effect in the frog skin and the rabbit urinary bladder electrical parameters. 3 - Evaluate the vasopressin effect in the frog skin short

circuit current. 4-Evaluate the amiloride effect in the frog and the rabbit urinary bladder. 5- Observe the pH influence in the frog urinary bladder transepithelial resistance.

METHODS
This is a prospective, analytic and experimental study. Animals were rabbits, rats and frogs. Control groups were the following isolated tissues: 1. Ten frog skins and urinary bladders, 2. Eight rabbit colon, urinary bladders and gallbladders, and 3. Five rat colon and urinary bladders. Cohort groups were the following: 1. Sixteen frog skins and urinary bladders and 2.Ten rabbit urinary bladders. Living frogs weighted between $100 - 200$ g, the rabbits $1.5 - 3$ kg and the rats $100 - 200$ g. Both solutions were at $20°C$. The eight cohort frog skins were exposed to 0.375 mM sodium, the other eight cohort frog skins were exposed to 100 mU/ml ADH in the serosal solution. Eight cohort frog urinary bladders were exposed to pH = 6 in the serosal solution and the other eight urinary bladders were exposed to 10 mM amiloride in the mucosal solution. The same electrical transepithelial parameters were measured from both control and cohort groups. Thirty minutes after the initial measures in the rabbit urinary bladder and the frog skin and urinary bladder control groups, these samples were exposed to the same manipulations as the cohort groups. **Transepithelial Electrical Parameters:** 1. Transepithelial voltage gradient (ΔV) is the voltage difference (mV) between the mucosal and the serosal solution. Fine tip electrodes are placed respectively in both solutions in order to measure the spontaneous voltage by a multimeter when less than 1 mV oscillations are constantly registered just 30 minutes after the open circuit is assembled. 2. Total transepithelial resistance (Rte) is calculated according to Ohm's Law (R=V/I) by registering the voltage change in the biological membrane when a 6 μA pulse current of one second duration is applied to the solutions, which is six times higher than the current injection reported in 1976 by Lewis & Diamond in their Electrical Methods. Unit resistance measure is expressed in kohm/cm^2 of surface area. The short-circuit current (Isc) is the current in the closed or shunted circuit that ocurrs when a zero or closed to zero transepithelial voltage is clamped by adding an outer $9 - 56$ V electromotive force and a potential divider that nulls the passive ionic transports in two symmetric solutions. The unit current measure is expressed in μA/cm^2. All measures in the control and the cohort groups were done thirty minutes after the open circuit was assembled.

Serrano, E.

The modified Ussing system consisted of two identical, cubic chambers built of acrilic material (size: 0.5 or 2 cm each side). The chamber size used in each control or cohort group depended on the biological sample size. The two chambers of five sides each were joined together, with the biological sample between them. A cyanoacrylate sealant was placed at the borders of the vacant side of each chamber to adhere the biological membrane and prevent the solution escape from each side of the system. Each chamber contained an electrolytic solution that occupied wether 125 μl in the 0.5 cm side chamber or 8 ml in the 2 cm side chamber. One solution contacted the apical side of the epithelium. This solution was called the mucosal solution. The other solution was nearer to the basolateral side of the epithelium, which was called the serosal solution. At the superior side of the chamber there were two apertures 3 mm in diameter. Five milimeters of a flexible plastic tube 5 cm long was introduced through one of each chamber orifices, which was also hermetically connected to a cylinder 1 – 2 cm diameter per 4 cm long. Two acrilic cylinders, each one connected to one chamber, contained the same electrolytical solution of the chambers and were opened at the top for receiving 3 L/min carbogen gas (95% O_2 and 5% CO_2) from a tank. The other orifice of each chamber was used to introduce not only the multimeter electrodes for voltage, current and temperature measures, but also the current inyection and/or the negative electrode in the closed circuit with the dry battery and the potential divider. Two digital multimeters model AVD-830D with 95% sensibility and 90% specificity were used in the electric circuit. Inyection current was provided by Grass SD9 stimulator which has 90% sensibility and 100% specificity. The voltimeter was located in parallel position with the tissue. In the closed electric circuit a dry 9 – 56 V battery was placed with the potential divider in series with the biological membrane, according to the particular sample transepithelial resistance and capacitance (Fig. 1: Closed Circuit in the Biological Assembling). The negative output of the battery was connected through a silver – silver chloride fine wire that ended in the chamber where the negative voltimeter electrode was handled. From a zero outer resistance, the potential divider was manipulated accurately so that as the resistance increase was obtained, the spontaneous membrane voltage dropped to zero. Then the short-circuit current was measured when the amperimeter was placed in series with the biological membrane. **Solutions:** Rabbit

Ringer (mM) 132 NaCl, pH 7.4, 7 KCl, 25 $NaHCO_3$, 2 $CaCl_2$, 1.2 $MgSO_4$, 0.375 NaH_2PO_4, 10 glucose; Rat Ringer Locke (mM) 130 NaCl, pH 7.4, 7 KCl, 25 $NaHCO_3$, 2 $CaCl_2$, 1.2 $MgSO_4$, 0.375 NaH_2PO_4, 10 glucose; Frog Ringer (mM) 102 NaCl, pH 7.4, 4 KCl, 5 $NaHCO_3$, 1 $CaCl_2$, 0.8 $MgSO_4$, 1 phosphate, 10 glucose; Ringer Coline (mM) 111.2 coline [17], pH 7.4, 5.8 KCl, 2 $CaCl_2$, 1.2 $MgSO_4$, 0.375 NaH_2PO_4, 10 glucose. **Drugs and Reagents:** Vasopressin 100 mU/ml, Amiloride 10 mM, HCl 0.1 N for serosal solution acidification to pH 6 and KCl 3 M for rabbits and rats sacrifices. **Dissections:** A.Rabbit and Rat. These animals were mentally depressed with 15 mg/kg of intraperitoneal xylazine, then they were sacrificed with 2cc of intracardiac KCl 3M. Urinary bladder and gallbladder were washed out vigorously three times with Ringer solution and placed each other separately in oxigenated solutions. The colon procedure was the same as the above but washed out five times to discard fecal content. The sample tissue had the least possible thickness, close to the epithelial thickness itself. The carefully connective and muscular tissues separations from the epithelia were done in the urinary bladder and the colon. B.Frog. The brain and spinal cord amphibian was traumatically, then a square of abdominal skin was taken out, 2.5 cm each side. The frog lobulated urinary bladder was also removed. **Total Sample and Epithelial Thicknesses Measures:** Each sample was fixed with buffered formalin and stained with hematoxilin and eosin. Eight different fields of the total samples and the epithelia thicknesses were measured with a 20x power vision light microscope. All epithelial average thicknesses were less than 35 μm and all the total sample thicknesses were less than 141 μm (Fig. 2). **Statistic Test:** I used the t student test with 0.01 and 0.05 level confidence not only for control group comparisons, but also for comparisons between control and cohort groups and between experimental and control periods of the control groups.
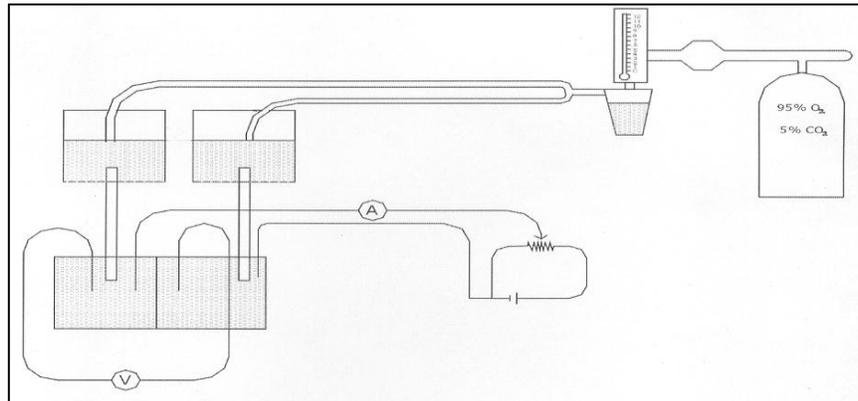
Serrano, E.

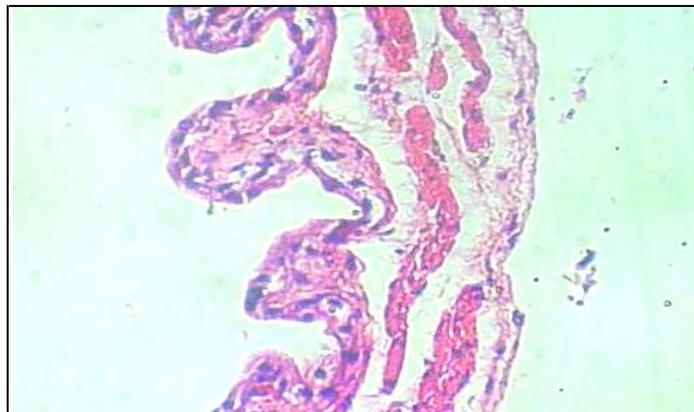Fig. 1. Closed Circuit in the Biological Assembling.

Fig. 2. Rabbit Urinary Bladder Total Thickness.

RESULTS

The frog skin and the rat urinary bladder had the highest average voltage gradients in their epithelia (34.4 mV and 28.1 mV respectively); instead the rat colon (1.8 mV) and the rabbit colon

(3.4 mV) had the lowest voltage gradients. The rat and rabbit urinary bladders had the highest transepithelial resistances (643.2 and 590.6 kohm/cm^2 respectively); instead the rabbit gallbladder had the lowest resistance (5.3 kohm/cm^2). Transepithelial resistances are mentioned in the decreasing order: rat urinary bladder > rabbit urinary bladder > frog skin > rabbit colon > frog urinary bladder > rat colon > rabbit gallbladder. The highest short-circuit current (Fig. 3) ocurred in the rabbit gallbladder epithelium (32.9 µA/cm^2), followed by the frog urinary bladder (24.7 µA/cm^2). The closed electric circuits of the rabbit colon and the rat urinary bladder control groups used the greatest external resistances for the short-circuit current determinations (302.5 and 352.2 kohm respectively). There were significant differences in voltage, resistance and short-circuit current parameters between the majority of the control groups comparisons for 0.01 level confidence, except in the following situations: 1-No voltage difference between rabbit and frog urinary bladders, 2-No voltage difference between rabbit urinary bladder and gallbladder, 3-No resistance and short-circuit curent differences between rabbit and rat urinary bladders. The low sodium experiment in the frog skin cohort group had lower spontaneous voltage, lower short-circuit current and higher resistance than the isotonic sodium in the frog skin control group for a 0.01 level confidence (Table 1). There were no statistical differences in the electric parameters between control and cohort rabbit urinary bladder groups in the low sodium experiment for a 0.01 level confidence. There was no voltage difference between the frog skin control and cohort groups in the vasopressin experiment, but resistance was significantly lower and short-circuit current higher in the cohort group. Both the amiloride and pH 6 experiments had lower spontaneous voltages and short circuit-currents in the frog urinary bladder cohort groups for a 0.01 level confidence. The short-circuit current and the transepithelial resistance in the rabbit urinary bladder cohort group were lower than the control group in the amiloride experiment for a 0.05 level condifence, but there was no difference in the spontaneous voltage. The electric parameters in ten control frog skins and urinary bladders and eight control rabbit urinary bladders were also measured thirty minutes after they were exposed to the experimental period. Five of the ten frog skins were exposed to low sodium and five were exposed to serosal vasopressin. Five of the ten frog urinary bladders were exposed to serosal pH 6 and five were exposed to mucosal amiloride. Four of the eight rabbit urinary bladders were exposed to

Serrano, E.

low sodium and four were exposed to amiloride. Contrary to the results in the cohort group, transepithelial resistance increased in the pH 6 and amiloride experimental periods of the control group (Table 2). Similarly the resistance increased in the amiloride experimental period of the rabbit urinary bladder control group. There were significant voltage and short-circuit current reductions in the low sodium experimental period in the frog skin control group, but resistance did not rise significantly. No significant voltage and short-circuit current rise were detected in the vasopressin frog skin experimental period, but resistance decreased for a 0.01 level confidence. The short-circuit currents and the voltages decreased in both the pH 6 and the amiloride manipulated frog urinary bladder in the experimental period of the control group. The resistance increased and the current decreased in the amiloride period of the control frog urinary bladders. Meanwhile the amiloride period did not change the rabbit urinary bladder parameters in the control group; neither did the low sodium period in the resistance and the voltage parameters. Instead the short-circuit current lowered in the low sodium period of the control rabbit urinary bladders. Amiloride in the experimental period decreased the short-circuit current in both the rabbit and the frog urinary bladders control groups for a 0.05 level confidence. Al epithelia (skin, urinary bladder, colon and gallbladder) had inverse relationships between transepithelial resistance and spontaneous voltage in the control periods.
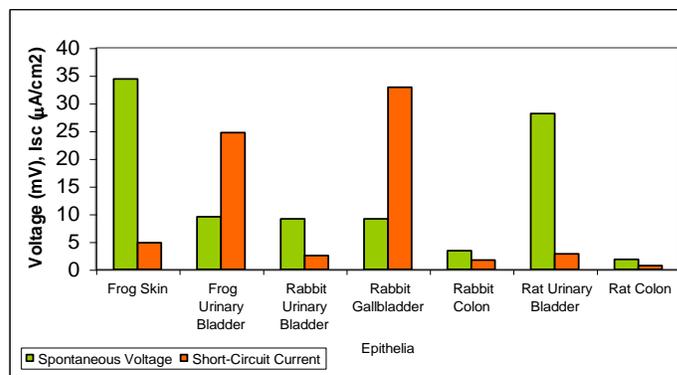

Fig. 3. Spontaneous Voltage and Short-Circuit Current in the Control Groups.

Table 1. Averages and Standard Deviations of Spontaneous Voltages, Transepithelial Resistances and Short-Circuit Currents in the Control and Cohort Groups.

Epithelia	Control Groups: Ringer Solutions				Cohort Groups: Ringer Choline			
	n	ΔV (mV)	R (kohm/cm^2)	Isc ($\mu A/cm^2$)	n	ΔV (mV)	R (kohm/cm^2)	Isc ($\mu A/cm^2$)
Frog Skin	10	34.4 +/- 11	82.8 +/- 9.0	4.8 +/- 1.1	8	13 +/- 4.9	93.1 +/- 3.8	3.1 +/- 0.7
Rabbit Urinary Bladder	8	9.1 +/- 2.6	590.6 +/- 107.9	2,5 +/- 0.4	5	6.4 +/- 1.8	586 +/- 78.6	1 +/- 0.2

Epithelia	Control Group				Cohort Group: Serosal ADH (100 mU/ml)			
	n	μV (mV)	R (kohm/cm^2)	Isc ($\mu A/cm^2$)	n	ΔV (mV)	R (kohm/cm^2)	Isc ($\mu A(cm^2)$)
Frog Skin	10	34.4 +/- 11	82.8 +/- 9.0	4.8 +/- 1.1	8	35.4 +/- 3.3	39.5 +/- 6	7.8 +/- 1.2

Epithelia	Control Group				Cohort Group: Serosal pH 6			
	n	ΔV (mV)	R (kohm/cm^2)	Isc ($\mu A/cm^2$)	n	ΔV (mV)	R (kohm/cm^2)	Isc ($\mu A/cm^2$)
Frog Urinary Bladder	10	9.5 +/- 2.3	35 +/-6.2	24.7 +/- 5.3	8	3.8 +/- 0.7	30.1 +/- 3.1	14.4 +/- 1.6

Epithelia	Control Groups				Cohort Groups: Mucosal Amiloride (10 mM)			
	n	ΔV (mV)	R (kohm/cm^2)	Isc ($\mu A/cm^2$)	n	ΔV (mV)	R (kohm/cm^2)	Isc ($\mu A/cm^2$)
Frog Urinary Bladder	10	9.5 +/- 2.3	35 +/-6.2	24.7 +/- 5.3	8	4.5 +/-1.4	31.6 +/- 5.8	12.4 +/- 2
Rabbit Urinary Bladder	8	9.1 +/- 2.6	590.6 +/- 107.9	2,5 +/- 0.4	5	9.5 +/- 2	405 +/- 135	1.8 +/- 0.5

Serrano, E.

Table 2. Averages +/- Standard Deviations of Spontaneous Voltages, Transepithelial Resistances and Short-Circuit Currents in the Control and Experimental Periods of the Control Groups.

		Control Period				Experimental Period		
	n	ΔV	Isc	Rte		ΔV	Isc	Rte
Epithelia		(mV)	(μA/cm2)	(kohm/cm2)	Manipulations	(mV)	(μA/cm2)	(kohm/cm2)
Frog Skin	5	32.8+/-8	3.6+/-0.6	90+/-15.1	Low Sodium	14.6+/-7.5	1+/-0.7	106.8+/-15.4
Frog Skin	5	35.1+/-12.5	5.9+/-2.1	75.6+/-8.7	Serosal ADH	52.1+/-7.3	9.1+/-1.7	41.4+/-6.9
Frog Urinary Bladder	5	10.6+/-3.3	28.1+/-5.4	42+/-9.1	Serosal pH 6	5.2+/-1.1	11.6+/-4.5	74+/-10.7
Frog Urinary Bladder	5	8.7+/-1.3	26.6+/-9.1	21.8+/-7.8	Amiloride	4.2+/-1	12.7+/-7.6	53.4+/-8.7
Rabbit Urinary Bladder	4	9.1+/-2.7	2+/-0.7	768.8+/-83.4	Low Sodium	3.8+/-3.1	0.5+/-0.6	787.5+/-83.1
Rabbit Urinary Bladder	4	9.1+/-2.6	3+/-0.5	412.5+/-154	Amiloride	4+/-3.2	2.2+/-0.3	424.8+/-156.1

DISCUSSION

This research first presents the in vitro epithelia electric parameters in amphibians and mammalians. Ussing found less than 91 mV spontaneous voltage and aproximately 40 μA/cm^2 current density in the frog skin. In the original assembling, he included agar bridges that connected the chambers to small cylinders filled with electrolytic solution. The cylinders contained calomel-KCl electrodes for the voltage and current measurements. Although the short-circuit current was lower in our research (4.8 +/- 1.1 μA/cm^2), the spontaneous voltage gradients in the frog skin were quiet similar in both studies (34.4 +/- 11.0 mV in the actual study). Lewis & Diamond (1976) found 20 – 75 mV spontaneous voltage, 1.5 – 20.5 μA/μF and 7000 – 40000 ohm-μF transepithelial resistance in the rabbit urothelium. Attending to membrane capacitance normatization in the rabbit urinary bladder, that is 1.36 μF/cm^2 due to mucosal folding, it means that these authors found currents and resistances about 2.0 – 27.9 μA/cm^2 and 5147 – 29411 ohm/cm^2 respectively. The actual research found lower spontaneous voltages and short-circuit currents (9.1 +/-2.6 mv and 2.5 +/- 0.4 μA /cm^2 respectively) and higher total membrane resistances (590.6 +/- 107.9 kohm/cm^2) than

Lewis & Diamond publications in 1976. The total transepithelial resistances obtained in the three species were in general greater than total resistances found in other publications. The total transepithelial resistances that were found in this research had magnitude orders similar to tight junction resistances. The total resistance in the rabbit urinary bladder control group in the actual study was 590.6 +/- 107.9 kohm/cm^2. The sodium substitution with choline decreased the short-circuit current in the frog skin and rabbit urinary bladder control groups in the experimental period, otherwise it was not significantly demonstrated in the rabbit urinary bladder cohort group of this research. As a matter of fact, it is described that the short-circuit current could be used as a sodium-potassium ATPase pump activity index (Tosteson 1989). The solvent flow through a biological membrane in the Ussing chamber could drag many ions. This fact could partly explain the Isc statistical differences between cohort groups and the experimental periods in the control groups. Considering the solvent drag, the flow ratio is not independent from the nature path, but directly proportional to the water volume flow rate and inversely related to the ion free diffusion quotation. The biological membrane areas used in the research, 0.25 cm^2 and 4 cm^2, were bathed at both sides by 125 μL and 8 mL respectively. As the short-circuit current and the total resistance parameters were expressed per superficial area, it permits the control and cohort groups comparisons, but it is possible that the different solvent flow volumes used according to the specific epithelium assembling determined the statistical differences mentioned above. The other factor that determines solvent drag is the integral describing the shape of the path the ion and the solvent have to follow. The sodium load effect in the apical membrane (Lyoussi et al., 1992; Palmer et al., 1988; Fuchs et al., 1977) and the intracellular sodium concentration will respectively change the epithelial sodium channel and the Na^+ - K^+ ATPase pump activities (Horisberger 1994). Under physiological conditions, intracellular sodium activates the pump with a half constant activation $K_{1/2}$ of $10 - 20$ mM and a Hill coefficient of $2 - 3$. It has been proposed that the intracellular sodium concentration recruits a latent pool of the pump subunits whose size is modulated by corticosteroids (Barlet-Bass et al., 1990). Lewis et al. (1984) pointed out by macroscopic transepithelial current fluctuation analysis that amiloride-sensitive apical sodium channels in the rabbit urinary bladder share characteristics with collecting ducts in the rabbit, as well as with the

Serrano, E.

amphibian skin and urinary bladder, otherwise no patch clamp measures have been made yet in isolated channels. A peak current that lowers to a steady state within a second is demonstrated when sodium concentration is increased in the mucosal side of the frog skin. These experiments prove that sodium extracellular concentration influences the channel activity independently from the sodium intracellular composition. This phenomenon represents a self-inhibition caused by the sodium union to the outer regulatory site of the channel. Amiloride is a pyrazinic diuretic that inhibits the sodium transport in the distal renal tubule (Baer et al., 1967), the frog urinary bladder (Bentley et al., 1968) , the frog skin and colon (Crabbé et al., 1968) and the rabbit urinary bladder (Frömter & Diamond 1972). As the sodium transport is inhibited by amiloride, the spontaneous voltage and the short current circuit are thus lowered by amiloride. The guanidinium group in the amiloride chemical structure blocks the Epithelial Sodium Channel (ENaC) at its outer entrance (Garty et al., 1988). In the present investigation, both the rabbit and the frog urinary bladder cohort and control groups exposed to amiloride had either lower or reduced short-circuit current and had either higher or augmented the transepithelial resistance for a 0.05 level confidence. Lewis (2000) stated the high amiloride sensitivity of the apical sodium transport at $2 - 3$ $\mu A/cm^2$. Our research found a higher short-circuit current in the frog urinary bladder control group (24.7 +/- 5,3 $\mu A/cm^2$), but the rabbit urinary bladder control group had a short-circuit current (2.5 +/- 0.4 $\mu A/cm^2$) within the Lewis current range proposal. Cox (1992) found that the amiloride sensitivity in the apical sodium channel depends on the amphibian age. Amiloride stimulated the short-circuit current in the frog tadpole skin, but inhibited it in the adult frog skin (Cox 1992, Mauro 1987).

Antidiuretic hormone increases the sodium transport and conductance in the frog skin, renal collecting duct and urinary bladder. Verrey (1994) found that vasopressin induces a bifasic short-circuit current in the *Xenopus laevis* distal nephron A6-C1 cells. Lewis and Diamond (1976) showed a low decrease by 22 +/- 8 % in the rabbit urinary bladder resistance, but no significant change in the short-circuit current with the addition of 100 mU ADH to the serosal bath. In the ADH frog skin exposed cohort group ocurred 52% less resistance and 62% more short-circuit current than the control group, with a slim

transepithelial voltage change. The actual research also found a 45% resistance decrease and a 54% short-circuit current increase during the vasopressin exposition in the experimental period of the frog skin control group. Lewis et al. (1976, 1984) found short-circuit current variations by changing the mucosal pH values: compared to pH 7.4, there was a 15% current increase at pH 8.4 and a 42% current decrease at pH 5.8. Our findings were the following: compared to pH 7.4 there was 60% less current and 29% less spontaneous voltage in the rabbit urinary bladder cohort group, and 35% less current and 62% less spontaneous voltage in the frog skin. The transepithelial resistences variations were less evident: compared to the pH 7.4 control groups, there was 12% less resistance in the frog skin and 1% less resistance in the rabbit urinary bladder cohort groups. The vasopressin´s transduction signal is as follows in the epithelia: it bounds to V_2 receptors in the basolateral cell membrane, then Gs protein is activated (Gs_α-ATP). Active Gs protein stimulates adenilateciclase, which increases the cAMP intracellular concentration. As the proteinkinase A fosforilates the ENaC aggregates, they are activated in the apical cell membrane, which will increase the sodium transport, the spontaneous voltage and the short-circuit current.

The hydrogen concentration has been implicated in the sodium-potassium ATPase pump activity since Lewis & Diamond publications in 1976. The pump is a current source in the basolateral side with one hundred charges per second per cycle or its equivalent 10^{-5} pA, which is 4 to 5 order magnitude lower than the ionic channel current. Multiple factors change the in vitro pump activity, particularly low oxygen tension, pH, osmolality and calcium concentration. Sodium enters the apical membrane following the electrochemical gradient into amiloride sensitive channels. The apical membrane of the rabbit urothelium has a –55 mV gradient voltage, negative the interior respect to mucosal solution, and a 7 mM chemical gradient when the sodium concentration in the mucosal solution is 120 mM. The basolateral sodium-potassium ATPase pump extrudes intracellular sodium at the time it introduces potassium to the cell. Potassium exits the basolateral membrane through selective potassium channels, which generate a –55 mV gradient voltage in the basolateral membrane. The rabbit urothelium pump kinetic has a 3 Na^+ and 2 K^+ stechiometry, with 14.2 mM sodium and 2.3 mM potassium Km. Hill coefficient for these ions are 2.8 and 1.8 respectively (Lewis 1983).

Serrano, E.

CONCLUSIONS

Most of the spontaneous voltage, total transepithelial resistance and the short-circuit current registered in the research were statistically different with 0.01 leve confidence. The frog and rabbit urinary bladders had different resistances and short-circuit currents between them. The same occured in all electric parameters between the frog skin and urinary bladder and between the rabbit colon and urinary bladder. Resistances and short-circuit currents were different between the rabbit urinary bladder and gallbladder. The rat colon and urinary bladder had different electric parameters in their epithelia, as well as the rat and rabbit colon are different. There were no statistical differences between the frog and rabbit urinary bladder voltage, between the rabbit urinary bladder and gallbladder voltage and between the rat and rabbit urinary bladder resistance and short-circuit current. Low sodium concentration in the baths decreased both the spontaneous voltage and the short-circuit current in the frog skin with a 0.01 level confidence, but transepithelial resistance did not change in the frog skin control group exposed to low sodium. Instead the frog skin transepithelial resistances in the cohort and the control group in the ADH experimental period were lower than the control groups not exposed to vasopressin. The frog skin short-circuit current increased in the control group experimental period exposed to vasopressin with a 0.01 level confidence. The frog urinary bladder cohort and control group in the experimental period had less spontaneous voltages when they were exposed to serosal pH 6. The same happened to the current in the control group exposed to pH 6, but the transepithelial resistance increased. The spontaneous voltage changed neither in the rabbit urinary bladder cohort group nor in the control group exposed to amiloride. The short-circuit current and the resistance did not significantly change in the rabbit urinary bladder control group exposed to amiloride, but the current decreased in the cohort group with a 0.05 level confidence. The external resistances in the cohort groups were not significantly different from control groups, except for the frog urinary bladder exposed to pH 6, in which the external resistance was 47% less than the control group. These results derived from a simple in vitro system that was electrically injected with an external electromotive force and a potential divider located in series with the biological membrane. It seems reasonable to repeat this methodology elsewhere, this time using the voltage clamp technique. Concomitantly, kinetic radiolabeled ion and water flows shall also be evaluated.

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