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Report

Electrical Analysis of Fresh, Excised Human Skin: A Comparison with Frozen Skin[1]

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Samples of human allograft skin prepared without freezing ("fresh skin") were found to have electrical and sodium ion transport properties which differed only slightly from those of skin which had been similarly treated but stored frozen ("frozen skin"). The fresh skin samples were less permeable to sodium ions during passive diffusion and less conductive than frozen skin at low current levels. They were more permselective for sodium versus chloride during constant-current iontophoresis and showed slightly more asymmetry in their current–voltage properties. Overall, the electrical behavior of the two tissues was similar enough to support the use of frozen tissue in iontophoresis studies. However, caution should be exercised when considering the use of frozen skin for applications, such as those based on electroosmosis, where the observed differences could have a major impact on the results.

KEY WORDS: iontophoresis; human skin; current–voltage characteristic; sodium ion transport; fresh skin; frozen skin.

INTRODUCTION

Drug ions passing from an iontophoretic patch into skin under the influence of an electric field must compete as charge carriers with one of the two major extracellular ions of the body, sodium or chloride. In order to make a priori estimates of the electrical current and voltage required to deliver a given amount of drug, the permeability of the membrane to sodium and chloride as a function of applied potential (i.e., the skin's current–voltage characteristic) must be known.

In an earlier study (1) we described the electrical and sodium ion transport properties of human allograft skin which had been slowly frozen in 10% glycerol to −150°C, stored frozen for up to 2 months, then thawed prior to use (herein called "frozen skin"). The DC resistance of frozen skin was found to be lower than that reported for human skin *in vivo,* yet the sodium ion permeabilities were similar to *in vivo* values. In this study we examined samples of allograft skin which had never been frozen ("fresh skin") in order more accurately to infer the effects of freezing on the tissue. The objective of the work was to validate the use of the more readily available frozen skin for iontophoresis studies.

MATERIALS AND METHODS

The apparatus and methods have been previously described (1,2). An outline of the procedures is given below.

Back skin from a male Caucasian donor was obtained from the Ohio Valley Skin and Tissue Center, Cincinnati, OH. The skin was procured with a dermatome set to 0.25 μm after the hair had been clipped and the skin washed. The skin was bathed in a solution of antibiotics (penicillin G, streptomycin) for 24 hr, then transported to the laboratory, where it was used immediately.

The skin was cut into small squares and mounted in either side-by-side iontophoresis cells (n = 12) or horizontally oriented passive diffusion cells (n = 24). The diffusional cross section for both types of cells was 0.7 cm^2. Both sides of the tissue were bathed in an isotonic saline buffer, which was Dulbecco's phosphate-buffered saline, pH 7.4, to which 0.02% (w/v) sodium azide had been added. For sodium ion transport measurements, the donor solution (epidermal side of skin) was spiked with 2.0 μCi/ml of $^{22}NaCl$, 99% radiochemical purity.

Protocol for Passive Diffusion Cells

After an overnight equilibration period with both sides of the skin immersed in buffer, the receptor solutions (5 ml) were replaced with fresh buffer and the donor solutions were removed and replaced with 0.5 ml of buffer containing $^{22}NaCl$. The receptor solutions were removed for radioactivity analysis at 2, 4, 6.5, 23, 47, and 71 hr postdose. Sodium ion permeability coefficients and diffusional time lags were determined from the cumulative penetration versus time curves by fitting straight lines through the data between 6.5 and 71 hr. Visual inspection and linear regression (mean r^2 = 0.988) showed the data to be highly linear over this range. The ratio of the slope of these lines to the sodium ion concentration in the donor solution yielded the permeability co-

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efficient, and their intercept with the time axis provided the time lag.

Protocol for Iontophoresis Cells

The following sequence of measurements was conducted: initial resistance determination (Day 1), current–voltage measurements (Days 2 and 3), $^{22}Na^+$ passive diffusion (Days 3–5), followed by a second resistance determination and $^{22}Na^+$ iontophoresis (Day 5).

The procedures were those of Reference 1 (Study 1), except that the passive diffusion stage was conducted for 45 hr. Skin resistance was determined by passing a direct current, I, of 10 μa (or current density, j, of 14 μA/cm^2) through each tissue for 5–10 sec, using a constant-current source. Additional current–voltage measurements were made using short bursts of direct current having alternating polarity and an intensity which first increased and then decreased in a stepwise manner. Three consecutive current regimens were imposed: 0 to ±10, 0 to ±50, and 0 to ±250 μA. A positive potential was defined as epidermis positive with respect to dermis. Completion of one sequence (20 readings) required about 10 min per sample. The current–voltage (j–V) data were characterized by the following equation (1):

$$V = (4aRT/F)\ \sinh^{-1}(bj) \qquad (1)$$

where R is the gas constant, T the absolute temperature, F the Faraday constant, and a and b are parameters which are properties of the membrane. Due to the constant-current methodology, voltage was treated as the dependent variable and current as independent. Following the passive permeability and resistance determinations, $^{22}Na^+$ iontophoresis was conducted for 6 hr at a constant current of 50 μA. Permeation samples were obtained every 2 hr during the iontophoresis treatment and analyzed for radioactivity using a gamma counter.

RESULTS

Skin Resistance and Sodium Ion Permeability Coefficients

Skin resistance values and passive sodium ion permeabilities are given in Table I. Summary parameters are shown in Table II, along with corresponding results from frozen skin (1). The fresh skin samples had a slightly higher median resistance and a 45% lower median permeability to Na^+ than the frozen skin samples. However, a statistical comparison of the results using the Wilcoxon rank sum test failed to show a significant difference in either R or k_p between the two tissues ($P > 0.2$, two-sided test).

Table I also shows that for 10 of 12 samples in the present study, electrical resistance decreased over the 4-day interval between mounting the skin and completing the passive sodium ion permeability measurements. The inverse of the final resistance values (i.e., the final conductance) showed a strong linear correlation with sodium ion permeability ($r^2 = 0.96$, excluding the very high permeability sample). This relationship was considerably tighter than that between initial conductance and Na^+ permeability ($r^2 = 0.24$). This change in the tissue properties over time explains our failure to observe a strong relationship between conductance and permeability in Ref. 1. Note that the resistance of one sample actually increased by more than 100% during the course of the study. We have observed similar resistance changes in other skin samples in subsequent experiments. The operating mechanism here is unknown; however, we offer the possibility that conductive shunts in such samples that are initially open to ion flow may swell shut as the tissue hydrates.

Diffusional Lag Times

Typical penetration versus time curves for the passive diffusion of Na^+ through skin in the side-by-side iontophoresis cells are shown in Fig. 1a. The mean lag time for achieve-

Table I. Sodium Ion Permeability Coefficients and Electrical Resistance for Fresh Excised Human Skin *in Vitro*

$k_p \times 10^6$ (cm/min)	R, kΩ[a]		$k_p \times 10^6$ (cm/min)	R, kΩ		$k_p \times 10^6$ (cm/min)	R, kΩ	
	Init.[b]	Final[c]		Init.	Final		Init.	Final
0.04[d]			0.23			1.49		
0.06			0.27	185	146	1.56	77	39
0.07			0.33			1.82		
0.07			0.35	253	161	1.83		
0.08			0.41			1.84		
0.10	238	198	0.44	87	118	1.85		
0.11	75	182	0.48	135	95	6.07		
0.12			0.49	127	107	6.16		
0.13			0.49	149	112	6.42		
0.14			0.50	135	81	12.83		
0.21			0.80			28.28		
0.23	193	126	0.89			51.40	26	12

[a] Effective resistance at 10 μA of 0.7-cm^2 samples.
[b] 15–60 min after mounting skin.
[c] Following k_p determination.
[d] k_p values without an associated value of R were determined in passive diffusion cells; the others were determined in iontophoresis cells following the current–voltage measurements.